RESEARCH **Issue Date:** 05/27/2014
Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 1R01AI110964-01
FAIN: R01AI110964

Principal Investigator(s):
PETER DASZAK, PHD

Project Title: Understanding the Risk of Bat Coronavirus Emergence

Aleksei
President
460 West 34th Street
17th Floor
New York, NY 100012317

Award e-mailed to: [redacted] (b) (6)

Budget Period: 06/01/2014 – 05/31/2015
Project Period: 06/01/2014 – 05/31/2019

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $666,442 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to ECOHEALTH ALLIANCE, INC. in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI110964. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Laura A. Pone
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 1R01AI110964-01

Award Calculation (U.S. Dollars)

Salaries and Wages	$167,708
Fringe Benefits	$54,168
Supplies	$21,400
Travel Costs	$35,918
Other Costs	$10,000
Consortium/Contractual Cost	$227,663

Federal Direct Costs	$516,857
Federal F&A Costs	$149,585
Approved Budget	$666,442
Federal Share	$666,442
TOTAL FEDERAL AWARD AMOUNT	$666,442

AMOUNT OF THIS ACTION (FEDERAL SHARE)	$666,442

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$666,442	$666,442
2	$630,445	$630,445
3	$611,090	$611,090
4	$597,112	$597,112
5	$581,646	$581,646

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93.855
EIN:	1311726494A1
Document Number:	RAI110964A

PMS Account Type:	P (Subaccount)
Fiscal Year:	2014

IC	CAN	2014	2015	2016	2017	2018
AI	8472350	$666,442	$630,445	$611,090	$597,112	$581,646

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M51C / **OC**: 414A / **Released**: (b) (6) 05/20/2014
Award Processed: 05/08/2014 01:52:21 PM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R01AI110964-01

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1R01AI110964-01

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.

b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI110964. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 1R01AI110964-01

THIS AWARD CONTAINS GRANT SPECIFIC RESTRICTIONS. THESE RESTRICTIONS MAY ONLY BE LIFTED BY A REVISED NOTICE OF AWARD.

RESTRICTION: This award is issued with the knowledge that subjects may be involved within the period of support, but definite plans were not set forth in the application as per 45 CFR 46.118. No human subjects may be involved in any project supported by this award until all requirements for Human Subjects research as identified in the PHS398/SF424 Instructions have been provided to and approved by NIH.

RESTRICTION: The present award is being made without a currently valid certification of IRB approval for this project with the following restriction: Only activities that are clearly severable and independent from activities that involve human subjects may be conducted pending the NIAID's acceptance of the certification of IRB review and approval.

No funds may be drawn down from the payment system and no obligations may be made against Federal funds for any research involving human subjects prior to the NIAID's notification to the grantee that the identified issues have been resolved and this restriction removed.

~~~~~~~~~

This award includes funds for subcontract/consortium activity with Wuhan Institute of Virology, CHINA and is budgeted as follows:

	-Yr 1	-Yr 2	-Yr 3	-Yr 4	-Yr 5
Total Direct Costs	$123,699	$128,718	$147,335	$147,335	$147,335
F&A Costs @ 8%(MTDC)	$9,896	$10,297	$11,787	$11,787	$11,787
TOTAL COSTS	$133,595	$139,015	$159,122	$159,122	$159,122

Consortiums are to be established and administered as described in the NIH Grants Policy Statement. This written agreement with the consortium must address the negotiated arrangements for meeting the scientific, administrative, financial, and reporting requirements for this grant.

~~~~~~~~~

This award includes funds for subcontract/consortium activity with East China Normal University, CHINA and is budgeted as follows:

	-Yr 1	-Yr 2	-Yr 3	-Yr 4	-Yr 5
Total Direct Costs	$87,100	$67,300	$50,108	$39,167	$14,850
F&A Costs @ 8%(MTDC)	$6,968	$5,384	$4,009	$3,133	$2,404
TOTAL COSTS	$94,068	$72,684	$54,117	$42,300	$32,454

Consortiums are to be established and administered as described in the NIH Grants Policy Statement. This written agreement with the consortium must address the negotiated arrangements for meeting the scientific, administrative, financial, and reporting requirements for this grant.

~~~~~~~~~

Select Agents:

Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:

NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.

When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.
~~~~~~~~~

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

o A list of the new and/or additional Agent(s) that will be studied;
o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Laura A. Pone
Email: [redacted] (b) (6) **Phone**: [redacted] (b) (6) **Fax**: 301-493-0597

Program Official: Erik J. Stemmy
Email: [redacted] (b) (6) **Phone**: [redacted] (b) (6)

SPREADSHEET SUMMARY
GRANT NUMBER: 1R01AI110964-01

INSTITUTION: ECOHEALTH ALLIANCE, INC.

Budget	Year 1	Year 2	Year 3	Year 4	Year 5
Salaries and Wages	$167,708	$167,708	$167,708	$167,708	$167,708
Fringe Benefits	$54,168	$54,168	$54,168	$54,168	$54,168
Supplies	$21,400	$19,250	$7,250	$7,000	$3,500
Travel Costs	$35,918	$35,918	$35,918	$35,918	$35,918
Other Costs	$10,000	$13,550	$11,050	$9,800	$9,400
Consortium/Contractual Cost	$227,663	$211,699	$213,239	$201,422	$191,576
TOTAL FEDERAL DC	$516,857	$502,293	$489,333	$476,016	$462,270
TOTAL FEDERAL F&A	$149,585	$128,152	$121,757	$121,096	$119,376
TOTAL COST	$666,442	$630,445	$611,090	$597,112	$581,646

Facilities and Administrative Costs	Year 1	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	44.1%	44.1%	44.1%	44.1%	44.1%
F&A Cost Base 1	$339,194	$290,594	$276,094	$274,594	$270,694
F&A Costs 1	$149,585	$128,152	$121,757	$121,096	$119,376

PI: **DASZAK, PETER**	Title: Understanding the Risk of Bat Coronavirus Emergence	
Received: 06/05/2013	FOA: PA11-260	Council: 01/2014
Competition ID: ADOBE-FORMS-B2	FOA Title: RESEARCH PROJECT GRANT (PARENT R01)	
1 R01 AI110964-01	Dual:	Accession Number: 3595101
IPF: 4415701	Organization: ECOHEALTH ALLIANCE, INC.	
Former Number:	Department:	
IRG/SRG: CRFS	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 499,993 Year 2: 499,469 Year 3: 499,978 Year 4: 499,953 Year 5: 499,974	Animals: Y Humans: Y Clinical Trial: N Current HS Code: (b) (4) HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Peter Daszak	EcoHealth Alliance, Inc.	PD/PI
ZhengLi Shi	Wuhan Institute of Virology	Co-Investigator
ShuYi Zhang	East China Normal University	Co-Investigator
Changwen Ke	CDC and Prevention of Guangdong Province	Co-Investigator
Jonathan Epstein	EcoHealth Alliance	Co-Investigator
Kevin Olival	EcoHealth Alliance	Co-Investigator
Parviez Hosseini	EcoHealth Alliance	Co-Investigator
XingYi Ge	Wuhan Institute of Virology	Co-Investigator
Guanjin Zhu	Guangdong Entomological Institute	Co-Investigator
Yun-Zhi Zhang	Yunnan Center for Disease Control	Co-Investigator

Additions for Review

Accepted Publication	News of manuscripts acceptance

OMB Number: 4040-0001
Expiration Date: 06/30/2011

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE

State Application Identifier

1. * TYPE OF SUBMISSION

☐ Pre-application ☐ Application ☒ Changed/Corrected Application

4. a. Federal Identifier GRANT11418218

b. Agency Routing Identifier

2. DATE SUBMITTED 06/05/2013

Applicant Identifier

5. APPLICANT INFORMATION

*** Organizational DUNS:** 0770900660000

* Legal Name: EcoHealth Alliance, Inc.

Department: Division:

* Street1: 460 West 34th Street

Street2: 17th Floor

* City: New York County / Parish:

* State: NY: New York Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 10001-2317

Person to be contacted on matters involving this application

Prefix: Dr. * First Name: Peter Middle Name:

* Last Name: Daszak Suffix:

* Phone Number: (b) (6) Fax Number: +1.212.380.4465

Email: (b) (6)

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN):* 311726494

7. * TYPE OF APPLICANT: M: Nonprofit with 501C3 IRS Status (Other than Institution of Higher Education)

Other (Specify):

Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION:

☒ New ☐ Resubmission

☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).

☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration

☐ E. Other (specify):

* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. * NAME OF FEDERAL AGENCY:

National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Understanding the Risk of Bat Coronavirus Emergence

12. PROPOSED PROJECT:

* Start Date 10/01/2013 * Ending Date 09/30/2018

*** 13. CONGRESSIONAL DISTRICT OF APPLICANT**

NY-010

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. * First Name: Peter Middle Name:

* Last Name: Daszak Suffix:

Position/Title: President

* Organization Name: EcoHealth Alliance, Inc.

Department: Division:

* Street1: 460 West 34th Street

Street2: 17th Floor

* City: New York County / Parish:

* State: NY: New York Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 10001-2317

* Phone Number: (b) (6) Fax Number: +1.212.380.4465

* Email: (b) (6)

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	3,362,338.00
b. Total Non-Federal Funds	0.00
c. Total Federal & Non-Federal Funds	3,362,338.00
d. Estimated Program Income	0.00

16. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☐ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☒ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious. or fraudulent statements or claims may subject me to criminal, civil, or administrative penalities. (U.S. Code, Title 18, Section 1001)

☒ *** I agree**

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or other Explanatory Documentation

Add Attachment | Delete Attachment | View Attachment

19. Authorized Representative

Prefix:	Mr.
* First Name:	Aleksei
Middle Name:	
* Last Name:	Chmura
Suffix:	
* Position/Title:	Authorized Organizational Representative
* Organization:	EcoHealth Alliance, Inc.
Department:	
Division:	
* Street1:	460 West 34th Street
Street2:	17th Floor
* City:	New York
County / Parish:	
* State:	NY: New York
Province:	
* Country:	USA: UNITED STATES
* ZIP / Postal Code:	10001-2317
* Phone Number:	(b) (6)
Fax Number:	+1.212.380.4465
* Email:	(b) (6)

* Signature of Authorized Representative	* Date Signed
Winifred Zubin	06/05/2013

20. Pre-application

Add Attachment | Delete Attachment | View Attachment

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OMB Number: 4040-0010
Expiration Date: 08/31/2011

Project/Performance Site Location(s)

Project/Performance Site Primary Location

☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: EcoHealth Alliance, Inc.
DUNS Number: 0770900660000
* Street1: 460 West 34th Street
Street2: 17th Floor
* City: New York
County:
* State: NY: New York
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 10001-2317
* Project/ Performance Site Congressional District: NY-010

Project/Performance Site Location 1

☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Wuhan Institute of Virology
DUNS Number: 5290274740000
* Street1: Xiao Hong Shan, No. 44
Street2: Wuchang District
* City: Wuhan
County:
* State:
Province: Hubei
* Country: CHN: CHINA
* ZIP / Postal Code: 430071
* Project/ Performance Site Congressional District:

Project/Performance Site Location a

☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: East China Normal University
DUNS Number: 4209454950000
* Street1: 3663 Zhongshan Beilu
Street2:
* City: Shanghai
County:
* State:
Province: Shanghai
* Country: CHN: CHINA
* ZIP / Postal Code: 200062
* Project/ Performance Site Congressional District:

Project/Performance Site Location(s)

Project/Performance Site Location 3

☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Yunnan Institute of Endemic Diseases Control and Prevention

DUNS Number:

* Street1: 33 Wenhua Road

Street2:

* City: Dali

County:

* State:

Province: Yunnan

* Country: CHN: CHINA

* ZIP / Postal Code: 650201

* Project/ Performance Site Congressional District:

Project/Performance Site Location a

☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Center for Disease Control and Prevention of Guangdong

DUNS Number:

* Street1: 176 Xigang Xilu

Street2:

* City: Guangzhou

County:

* State:

Province: Guangdong

* Country: CHN: CHINA

* ZIP / Postal Code: 5103000

* Project/ Performance Site Congressional District:

Additional Location(s)

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? [X] Yes [ ] No

1.a If YES to Human Subjects

Is the Project Exempt from Federal regulations? [ ] Yes [X] No

If yes, check appropriate exemption number. [ ] 1 [ ] 2 [ ] 3 [ ] 4 [ ] 5 [ ] 6

If no, is the IRB review Pending? [X] Yes [ ] No

IRB Approval Date:

Human Subject Assurance Number:

2. * Are Vertebrate Animals Used? [X] Yes [ ] No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? [ ] Yes [X] No

IACUC Approval Date: 10/15/2010

Animal Welfare Assurance Number A3433-01

3. * Is proprietary/privileged information included in the application? [ ] Yes [X] No

4.a. * Does this project have an actual or potential impact on the environment? [ ] Yes [X] No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? [ ] Yes [ ] No

4.d. If yes, please explain:

5. * Is the research performance site designated, or eligible to be designated, as a historic place? [ ] Yes [X] No

5.a. If yes, please explain:

6. * Does this project involve activities outside of the United States or partnerships with international collaborators? [X] Yes [ ] No

6.a. If yes, identify countries: China

6.b. Optional Explanation:

7. * Project Summary/Abstract 1234-NIAID_COV_Project_Summary2013.pd Add Attachment Delete Attachment View Attachment

8. * Project Narrative 1235-NIH_COv_Project_Narrative.pdf Add Attachment Delete Attachment View Attachment

9. Bibliography & References Cited 1236-NIAID_COV_2013_Bibliography.pdf Add Attachment Delete Attachment View Attachment

10. Facilities & Other Resources 1237-FACILITIES_AND_OTHER_RESOURCES.p Add Attachment Delete Attachment View Attachment

11. Equipment Add Attachment Delete Attachment View Attachment

12. Other Attachments Add Attachments Delete Attachments View Attachments [ ]

Project Summary

This project will examine the risk of future coronavirus (CoV) emergence from wildlife using in-depth field investigations across the human-wildlife interface in China, molecular characterization of novel CoVs and host receptor binding domain genes, mathematical models of transmission and evolution, and *in vitro* and *in vivo* laboratory studies of host range. Zoonotic CoVs are a significant threat to global health, as demonstrated with the emergence of pandemic severe acute respiratory syndrome coronavirus (SARS-CoV) in China in 2002, and the recent and ongoing emergence of Middle East Respiratory Syndrome (MERS-CoV). Bats appear to be the natural reservoir of these viruses, and hundreds of novel bat-CoVs have been discovered in the last two decades. Bats, and other wildlife species, are hunted, traded, butchered and consumed across Asia, creating a largescale human-wildlife interface, and high risk of future emergence of novel CoVs. This project aims to understand what factors increase the risk of the next CoV emerging in people by studying CoV diversity in a critical zoonotic reservoir (bats), at sites of high risk for emergence (wildlife markets) in an emerging disease hotspot (China). The three specific aims of this project are to:

1. Assess CoV spillover potential at high risk human-wildlife interfaces in China. This will include quantifying the nature and frequency of contact people have with bats and other wildlife; serological and molecular screening of people working in wet markets and highly exposed to wildlife; screening wild-caught and market sampled bats from 30+ species for CoVs using molecular assays; and genomic characterization and isolation of novel CoVs.
2. Develop predictive models of bat CoV emergence risk and host range. A combined modeling approach will include phylogenetic analyses of host receptors and novel CoV genes (including functional receptor binding domains); a fused ecological and evolutionary model to predict host-range and viral sharing; and mathematical matrix models to examine evolutionary and transmission dynamics.
3. Test predictions of CoV inter-species transmission. Predictive models of host range (i.e. emergence potential) will be tested experimentally using reverse genetics, pseudovirus and receptor binding assays, and virus infection experiments across a range of cell cultures from different species and humanized mice.

PROJECT NARRATIVE

Most emerging human viruses come from wildlife, and these represent a significant threat to global public health and biosecurity - as demonstrated by the SARS coronavirus pandemic of 2002-03 and an ongoing SARS-like epidemic in the Middle East. This project seeks to understand what factors allow animal Coronaviruses to evolve and jump into the human population by studying virus diversity in a critical group of animals (bats), at sites of high risk for emergence (wildlife markets) in an emerging disease hotspot (China).

FACILITIES AND OTHER RESOURCES

EcoHealth Alliance, New York, USA (Peter Daszak, Jon Epstein, Parviez Hosseini, and Kevin Olival)
EcoHealth Alliance is a 40-year old scientific research NGO that specializes in multidisciplinary research on the causes, origins and spread of zoonotic emerging diseases. EcoHealth Alliance scientists have been working on the emergence of Nipah & Hendra virus, SARS CoV, surveillance for zoonotic agents in wildlife, and spatial modeling for over 15 years, and on modeling of infectious disease emergence and spread for over a decade. EcoHealth Alliance is based in New York City with (b) (4) square feet of office space including a meeting room and basic laboratory – freezer storage and light microscopy. The scientific staff (25 core scientists, 100+ field staff) is supported by a core admin staff of 11 which is available for work on this project and is funded through core funds.

EcoHealth Alliance is equipped with 35 networked PCs including an NIH ARRA-funded International LifeSize Video Conferencing facility. High-speed video conferencing facilities have been installed with key international collaborators in 2011. EcoHealth Alliance has access to a 24-7 server, server support, and all required software including ArcGIS ArcINFO, MatLab, SPSS, R, Microsoft Office, and Adobe CS5 running on both Apple and Windows Operating Systems. Additionally we have a four-processor, public IP addressed Linux and an eight-processor Mac Pro Server - each with 4TB hard drives, which in combination can be used for intensive computational modeling and database processing by all the grantees. Access to the cloud (Amazon) is provided by core funding to EHA.

EcoHealth Alliance is the headquarters of a series of global networks that provide exceptional leverage for the core scientists: 1) The USAID EPT PREDICT consortium. This group conducts human and wildlife surveillance for high-risk pathogens in 24 countries including China. Partners include Dr Ian Lipkin's Center for Infection and Immunity at Columbia University (pathogen discovery), UC Davis, The Smithsonian, GVFI and WCS; 2) The One Health Alliance of South Asia. This is a Rockefeller Foundation funded transboundary disease surveillance program; 3) The EcoHealth Alliance Partners: A global partnership of leading wildlife and global health researchers in tropical and subtropical countries. This gives us unique access to working on-the-ground in countries where surveillance is difficult, such as China, where our group has proven capacity to export samples from; 4) The Consortium for Conservation Medicine: A unique collaborative institution linking Johns Hopkins Bloomberg School of Public Health, Tufts University School of Veterinary Medicine, The University of Pittsburgh Graduate School of Public Health, The University of Wisconsin-Madison Nelson Institute for Environmental Studies, The USGS National Wildlife Health Center, and EcoHealth Alliance. The CCM provides access to hundreds of high caliber scientists, their facilities,

and their students at 6 leading institutes of public health, veterinary medicine, and environmental science in the USA.

East China Normal University, **Shanghai, China** (Shuyi Zhang and Guangjian Zhu)
Dr. Zhang is Dean of the Institutes for Advanced Interdisciplinary Research at East China Normal University. Over (b)(4) square metres is allocated to his research group at ECNU. The lab is fully capable of carrying out molecular, protein, epidemic disease and evolution research. Experimental equipment includes: Roche 454 (GS FLX Titanium System), Bioinformatics Computer Server, Multi-Channel Neurophysiology Workstation TDT, PCR Amplifier, Real time PCR Amplifier, Electrophoresis, Ultra-low Temperature Freezer, Centrifuge, UV-Visible Spectrophotometer, Two-dimensional Electrophoresis, Vertical Electrophoresis System, Incubator, Clean Bench, and Class II-Biosafety Cabinet, Hybridization Oven, Water PurificationSystem, and Shaker.

Wuhan Institute of Virology, Wuhan, China (Zhengli Shi and Xingyi Ge)
The Shi laboratory includes 4 rooms totaling (b)(4), one equipped with two CO2 incubators for tissue culture, one equipped facilities including with high speed centrifuge, 2 -20°C, 3 -80°C freezers, 2 PCR machines, 1 ELISA plate reader, one for molecule diagnosis equipped with two biosafety cabinets, and one normal laboratory equipped various small equipment items (mini-centrifuges, gel electrophoresis units, circulating adjustable water baths, and heat blocks). Also available to Dr. Shi's group is a fully equipped biosafety level 3 laboratory, a newly opened BLS-4 laboratory (the first in China) and Institute-supported facility center, which houses full-time staff and equipment for electronic microscopy, ultracentrifugation, confocal microcopy, and sequencing machine.

The Wuhan Institute of Virology is China's premier institute for virological research. It consists of three research departments and one center: Department of Molecular Virology, Department of Bio-control, Department of Analytical Biochemistry and Biotechnology, and the Virus Resource and Bioinformation Center of China. It also has the Key Laboratory of Molecular Virology of CAS, the Joint-laboratory of Invertebrate Virology, a HIV Pre-screening Lab and Hubei Engineering and Technology Research Center for Viral Diseases. The institute is further divided into 14 research groups, one of which is run by Dr Zhengli Shi. The supporting system of the institute consists of an Analytical Equipment Center, an Experimental Animal Center, an Editorial Office of "Virologica Sinica"and an Computer Network Center. The virus resource and bio-information center of China contains the largest virus bank in Asia, curating around 800 viral strains.

The Institute collaborates with the World Health Organization (WHO), universities and research institutes in more than 30 counties and regions including EcoHealth Alliance in the USA. There are 14 professors, 36 associated professors, 47 assistant professors conducting research on virology and five of these have been awarded honors in the "Hundred Talents Project". The institute has built a BSL-3 lab and a 600 m2 experimental animal center. In 2013, the first BSL-4 lab in China was opened at this

Institute in a purpose-built facility which has been designed with the assistance of the US Centers for Disease Control and the Pasteur Institute.

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. | * First Name: Peter | Middle Name:
* Last Name: Daszak | Suffix:
Position/Title: President | Department:
Organization Name: EcoHealth Alliance, Inc. | Division:
* Street1: 460 West 34th Street
Street2: 17th Floor
* City: New York | County/ Parish:
* State: NY: New York | Province:
* Country: USA: UNITED STATES | * Zip / Postal Code: 10001-2317
* Phone Number: (b) (6) | Fax Number: +1.212.380.4465
* E-Mail: (b) (6)

Credential, e.g., agency login: (b) (6)

*** Project Role:** PD/PI | **Other Project Role Category:**

Degree Type: Ph.D.
Degree Year: 1994

***Attach Biographical Sketch** 1244-Peter_Daszak_NIH_biosket | Add Attachment | Delete Attachment | View Attachment
Attach Current & Pending Support | Add Attachment | Delete Attachment | View Attachment

PROFILE - Senior/Key Person 1

Prefix: Dr. | * First Name: ZhengLi | Middle Name:
* Last Name: Shi | Suffix:
Position/Title: Senior Scientist | Department:
Organization Name: Wuhan Institute of Virology | Division:
* Street1: Xiao Hong Shan, no. 44
Street2: Wuchang District
* City: Wuhan | County/ Parish:
* State: | Province: Hubei
* Country: CHN: CHINA | * Zip / Postal Code: 430071
* Phone Number: (b) (6) | Fax Number: +86-27-87198072
* E-Mail: (b) (6)

Credential, e.g., agency login:

*** Project Role:** Co-Investigator | **Other Project Role Category:**

Degree Type: Ph.D.
Degree Year: 2000

***Attach Biographical Sketch** 1245-SHI_Zhengli_Biosketch_20 | Add Attachment | Delete Attachment | View Attachment
Attach Current & Pending Support | Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 2

Prefix: Dr.
* First Name: ShuYi
Middle Name:
* Last Name: Zhang
Suffix:
Position/Title: Dean
Department:
Organization Name: East China Normal University
Division:
* Street1: B319, Science Building 3663
Street2: North Zhongshan Road
* City: Shanghai
County/ Parish:
* State:
Province: Shanghai
* Country: CHN: CHINA
* Zip / Postal Code: 200062
* Phone Number: [redacted] (b) (6)
Fax Number:
* E-Mail: [redacted] (b) (6)

Credential, e.g., agency login:

*** Project Role:** Co-Investigator
Other Project Role Category:

Degree Type: Ph.D.
Degree Year: 1994

***Attach Biographical Sketch** 1246-Zhang_Shuyi_Biosketch_KS | Add Attachment | Delete Attachment | View Attachment
Attach Current & Pending Support | Add Attachment | Delete Attachment | View Attachment

PROFILE - Senior/Key Person 3

Prefix: Dr.
* First Name: Changwen
Middle Name:
* Last Name: Ke
Suffix:
Position/Title: Director
Department:
Organization Name: CDC and Prevention of Guangdong Province
Division:
* Street1: Xing Gang West Road, no. 176
Street2:
* City: Guangzhou
County/ Parish:
* State:
Province: Guangdong
* Country: CHN: CHINA
* Zip / Postal Code: 510300
* Phone Number: [redacted] (b) (6)
Fax Number:
* E-Mail: [redacted] (b) (6)

Credential, e.g., agency login:

*** Project Role:** Co-Investigator
Other Project Role Category:

Degree Type: Ph.D.
Degree Year: 2001

***Attach Biographical Sketch** 1247-Biosketch_ChangWenKe_COV | Add Attachment | Delete Attachment | View Attachment
Attach Current & Pending Support | Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 4

Prefix: Dr. * First Name: Jonathan Middle Name: H
* Last Name: Epstein Suffix:
Position/Title: Associate Vice President Department: Conservation Medicine
Organization Name: EcoHealth Alliance Division:
* Street1: 460 W34th Street
Street2: 17th Floor
* City: New York County/ Parish:
* State: NY: New York Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 10001-2317
* Phone Number: [redacted] (b) (6) Fax Number: +1.212.380.4465
* E-Mail: [redacted] (b) (6)

Credential, e.g., agency login:

*** Project Role:** Co-Investigator **Other Project Role Category:**

Degree Type: DVM
Degree Year: 2002

***Attach Biographical Sketch** 1248-Epstein_BioSketch_NIH _$ Add Attachment Delete Attachment View Attachment
Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

PROFILE - Senior/Key Person 5

Prefix: Dr. * First Name: Kevin Middle Name:
* Last Name: Olival Suffix:
Position/Title: Senior Research Scientist Department:
Organization Name: EcoHealth Alliance Division:
* Street1: 460 W34th Street
Street2: 17th Floor
* City: New York County/ Parish:
* State: NY: New York Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 10001-2317
* Phone Number: [redacted] (b) (6) Fax Number: +1.212.380.4465
* E-Mail: [redacted] (b) (6)

Credential, e.g., agency login:

*** Project Role:** Co-Investigator **Other Project Role Category:**

Degree Type: Ph.D.
Degree Year: 2008

***Attach Biographical Sketch** 1249-Olival_biosketch_NIAID_C Add Attachment Delete Attachment View Attachment
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RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 6

Prefix: Dr.
* First Name: Parviez
Middle Name:
* Last Name: Hosseini
Suffix:
Position/Title: Senior Research Scientist
Department:
Organization Name: EcoHealth Alliance
Division:
* Street1: 460 W 34th Street
Street2: 17th Floor
* City: New York
County/ Parish:
* State: NY: New York
Province:
* Country: USA: UNITED STATES
* Zip / Postal Code: 10001-2317
* Phone Number: [redacted] (b) (6)
Fax Number: +1.212.380.4465
* E-Mail: [redacted] (b) (6)

Credential, e.g., agency login:

*** Project Role:** Co-Investigator
Other Project Role Category:

Degree Type: Ph.D.
Degree Year: 2002

***Attach Biographical Sketch** 1250-HOSSEINI_Biosketch_COV20 | Add Attachment | Delete Attachment | View Attachment
Attach Current & Pending Support | Add Attachment | Delete Attachment | View Attachment

PROFILE - Senior/Key Person 7

Prefix: Dr.
* First Name: XingYi
Middle Name:
* Last Name: Ge
Suffix:
Position/Title: Assistant Researcher
Department: Department of Emerging Viruses
Organization Name: Wuhan Institute of Virology
Division:
* Street1: Xiao Hong Shan, no. 44
Street2: Wuchang District
* City: Wuhan
County/ Parish:
* State:
Province: Hubei
* Country: CHN: CHINA
* Zip / Postal Code: 430071
* Phone Number: [redacted] (b) (6)
Fax Number:
* E-Mail: [redacted] (b) (6)

Credential, e.g., agency login:

*** Project Role:** Co-Investigator
Other Project Role Category:

Degree Type: Ph.D.
Degree Year: 2011

***Attach Biographical Sketch** 1251-GE_XingYi_Biosketch_2013 | Add Attachment | Delete Attachment | View Attachment
Attach Current & Pending Support | Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 8

Prefix: Dr.
* First Name: Guanjin
Middle Name:
* Last Name: Zhu
Suffix:
Position/Title: Assistant Researcher
Department:
Organization Name: Guangdong Entomological Institute
Division:
* Street1: Room 1707, Building 622, 3663 Zhongshanbei Rd
Street2: Putuo District
* City: Shanghai
County/ Parish:
* State:
Province:
* Country: CHN: CHINA
* Zip / Postal Code: 200026
* Phone Number: [redacted] (b) (6)
Fax Number:
* E-Mail: [redacted] (b) (6)

Credential, e.g., agency login:

*** Project Role:** Co-Investigator
Other Project Role Category:

Degree Type: Ph.D.
Degree Year: 2012

***Attach Biographical Sketch** 1252-Zhu_Gunagjian_Biosketch_ Add Attachment Delete Attachment View Attachment
Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

PROFILE - Senior/Key Person 9

Prefix: Dr.
* First Name: Yun-Zhi
Middle Name:
* Last Name: Zhang
Suffix:
Position/Title: Head of Infectious Disease Surveillance
Department:
Organization Name: Yunnan Center for Disease Control
Division:
* Street1: 33 Wenhua Road
Street2:
* City: Dali City
County/ Parish:
* State:
Province: Yunnan
* Country: CHN: CHINA
* Zip / Postal Code: 671000
* Phone Number: [redacted] (b) (6)
Fax Number:
* E-Mail: [redacted] (b) (6)

Credential, e.g., agency login:

*** Project Role:** Co-Investigator
Other Project Role Category:

Degree Type: Ph.D.
Degree Year: 2010

***Attach Biographical Sketch** 1253-Biosketch_YunZhiZhang_CO Add Attachment Delete Attachment View Attachment
Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Peter Daszak	POSITION TITLE President & Chief Scientist
eRA COMMONS USER NAME (credential, e.g., agency login) (b) (6)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Bangor University (UK)	BSc. (hons)	07/86	Zoology
University of East London (UK)	Ph.D	03/93	Infectious Diseases

A. Personal Statement

The goal of the proposed research is to investigate the ecology, evolutionary biology and transmission dynamics of bat coronaviruses at the human-wildlife interface. Specifically, we will conduct field studies in China to obtain high quality samples from bats, and identify, characterize and isolate known and novel coronaviruses. We will analyze the patterns of coronavirus transmission among bats and other wildlife, and the risk of spillover to humans. I have been working on the dynamics of emerging viral diseases from wildlife for over 15 years, and have the proven scientific vision, leadership and capacity to lead this team and test the hypotheses laid out here. Since working at the CDC Pathology Activity in 1998 during the Nipah virus outbreak, I have specialized in the ecology of viruses emerging from bats. Under my first Nipah virus R01, I developed a multidisciplinary approach combining fieldwork, phylogenetics, virology, and mathematical modeling to isolate and characterize NiV from bats, analyze transmission dynamics, and identify the cause of its emergence. In 2001, I became director of a research program at a New York-based scientific research NGO. This allowed me to expand my research globally, and in 2005, working with current co-investigators Drs Zhang and Zhengli, we were the first team to identify and characterize SARS-like coronaviruses in bats. I have consolidated this work as PI of: 1) a NIAID R01 to conduct pathogen discovery in bats, and map bat viral diversity; 2) a renewal to my Nipah virus R01 focused on the emergence of NiV in Bangladesh; and 3) a large USAID project (PREDICT) to identify new pathogens in wildlife from emerging disease 'hotspot' regions. The current application builds on this work and leverages my group's unique partnership in China, where we have proven capacity to conduct disease surveillance in humans and wildlife in the markets where SARS emerged, and where we have collaborated at a high level for 12 years. I have a proven record of leading multidisciplinary research teams on emerging viral pathogens from wildlife and have the leadership skills, institutional capacity and network to deliver successful outcomes in the current proposed work.

B. Positions and Honors

Positions and Employment

1993-8	Senior Faculty Research Scientist, Kingston University
1998	Guest Researcher, Centers for Disease Control and Prevention (CDC)
1999-2001	Faculty Research Scientist, University of Georgia
2001-	Adjunct Faculty, Tufts Univ. Sch. Veterinary Med.; Univ. Georgia; Columbia Univ.
2001-9	Executive Director, Consortium for Conservation Medicine, EcoHealth Alliance, New York
2009-	President & Chief Scientist, EcoHealth Alliance New York.

Other Experience and Professional Membership

Keynote speaker Merieux Foundation Conference on Emerging paramyxoviruses, France (2000); UN Millenium Ecosystem Assessment: Lead Author, human infectious diseases (2006); NIH: ad hoc member, ZRG1 IDM-G 90 study section: Virology, Biodefense & Emerg. Diseases (2003-5); Editorial Board, Conservation Biology (Blackwell); Founding Co-Editor *EcoHealth* (Springer) (2004-10); NAS – Committee Member, Future Needs in Veterinary Research (2004-5); DIVERSITAS (UNESCO-ICSU): Member of Scientific Committee (2004-11; Treasurer 2007-11); NIAID: Steering Committee, workshop on virus-host shifts & emergence of new pathogens (2005); Australian Biosecurity Cooperative Research Center: International Standing Advisory Committee (2005-10); NIH: ad hoc member, ZRG1 IRAP-Q study section (infectious diseases, epidemiology) (2005-7); International EcoHealth Association: Founding board of directors, Treasurer (2006-11); CDC: ad hoc member, ZCD1 SGI, 09PAR07-231, R36 Research Dissertation Awards (2007); European CDC: Keynote speaker, future infectious disease threats (2008); NAS-IOM Committee Member, Global capacity for EID surveillance (2008-9); Scientific Advisory Board, NIAID Center of Excellence, avian influenza (CRISAR), UCLA (2008-9); Reviewer IOM report on Infectious Disease Movements in a Borderless World (2009); NIAID: Steering Committee, workshop on viruses from bats (2009); NAS-IOM Participant, workshop on H1N1, Committee on Emerging Microbial Threats (2009); NIH: ZRG1 IRAP-Q Review panel ARRA Challenge grants (2009); Organizing Committee, 1st International One Health Symposium, Australia (2010); Member, Council of Advisors One Health Commission (2010-); Editor-in-Chief, *EcoHealth* (2010-); Scientific Advisory Board, Oxford Univ. Clinical Research Unit, Vietnam (2010-); Member of IOM Forum on Microbial Threats 2010-; Steering Committeee, NIAID Workshop on Arboviruses 2011; Organizer IOM Forum on Microbial Threats briefing on MERS-CoV 2013.

Honors

Meritorious service award, CDC (1999); CSIRO silver medal for collaborative research (2000); Honored by the naming of a new species of centipede, *Cryptops daszaki* (J Nat Hist 2002; 36: 76-106) (2002); ISI Fast-breaking paper (2002); CBS 60 Minutes documentary on Nipah virus research; 6th Annual Lecturer, Medicine & Humanities, Texas A&M (2003); Editor's choice, *Science* (2006); Zayed International Prize for the Environment (2nd) (2006); Finalist, Director's Pioneer Award (2007); Discovery Channel documentary on Nipah virus research, Bangladesh (2008); Presidential Lecturer, University of Montana (2008) ; Elected member of the Cosmos Club 2012; Honored by the naming of a new species of parasite, *Isospora daszaki* (*Parasitol. Res.* 2013; 111:1463-1466) (2012); Awarded the Hsu-Li Distinguished Lectureship in Epidemiology (2013).

C. Peer-reviewed publications (selected from over 190+); * = Corresponding author

Most relevant to the current application

1. **Daszak P**, Cunningham AA, Hyatt AD (2000). Emerging infectious diseases of wildlife - threats to biodiversity and human health. Science 287: 443-449
2. Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, Wang H, Crameri G, Hu Z, Zhang H, Zhang J, McEachern J, Field H, **Daszak P**, Eaton BT, Zhang S & Wang L-F (2005). Bats are natural reservoirs of SARS-like coronaviruses. Science 310: 676-679.
3. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, and **Daszak P* (Corresponding Author)**. (2008). Global trends in emerging infectious diseases. Nature 451:990-993
4. Keesing F, Belden LK, **Daszak P**, Dobson A, Harvell CD, Holt RD, Hudson P, Jolles A, Jones KE, Mitchell CE, Myers SS, Bogich T & Ostfeld RS. (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature 468:647-652.
5. Morse SS, Mazet JAK, Woolhouse M, Parrish CR, Carroll D, Karesh WB, Zambrana-Torrelio C, Lipkin WI, **Daszak P* (Corresponding Author)** (2012). Prediction and prevention of the next pandemic zoonosis. Lancet 380:1956-1965.
6. **Daszak P** (2012). Anatomy of a pandemic Lancet 380: 1883-1884.

7. Quan P-L, Firth C, Conte JM, Williams SH, Zambrana-Torrelio C, Anthony SJ, Ellison JA, Gilbert AT, Kuzmin IV, Niezgoda M, Osinubi MOV, Recuenco S, Markotter W, Breiman R, Kalemba L, Malekani J, Lindblade KA, Rostal MK, Ojeda-Flores R, Suzan G, Davis LB, Blau DM, Ogunkoya AB, Castillo DAA, Moran D, Ngam S, Akaibe D, Agwanda B, Briese T, Epstein JH, **Daszak P**, Rupprecht CE, Holmes EC, Lipkin WI. (2013). Bats are a major natural reservoir for hepaciviruses and pegiviruses. PNAS Published ahead of print April 2013.
8. Anthony SJ, Ojeda-Flores R, Rico-Chávez O, Navarrete-Macias I, Zambrana-Torrelio CM, Rostal MK, Epstein JH, Tipps T, Liang E, Sanchez-Leon M, Sotomayor-Bonilla J, Ávila R, Medellín RA, Goldstein T, Suzán G, Daszak P, Lipkin WI. (2013). Coronaviruses in bats from Mexico **Journal of General Virology** Online First.
9. Anthony SJ, Epstein JH, Murray KA, Navarrete-Macias I, Zambrana-Torrelio CM, Solovyov A, Ojeda-Flores R, Arrigo NC, Islam A, Ali Khan S, Hosseini P, Bogich TL, Olival KJ, Sanchez-Leon MD, Karesh W, Goldstein T, Luby SP, Morse SS, Mazet JAK, **Daszak P*(Co-corresponding Author)**, Lipkin WI, Estimating viral diversity in Bats. *PNAS* in review.
10. [redacted] (b) (6)

Additional recent publications of importance to the field (from 190+ total)

1. Cui J, Han N, Streicker D, Li G, Tang X, Shi Z, Hu Z, Zhao G, Fontanet A, Yi G, Wang L, Jones G, .Field HE, **Daszak P* (Corresponding Author)** & Zhang, S. (2007) Evolutionary relationships between bat coronaviruses and their hosts. *Emerg. Infect. Dis.* 13: 1526-1533
2. Epstein JH, Prakash V, Smith CS, **Daszak P**, McLaughlin AB, Meehan G, Field HE, Cunningham AA (2008). Henipavirus infection in fruit bats (*Pteropus giganteus*), India. *Emerg. Infect. Dis.* 14: 1309-1311.
3. Smith KF, Behrens M, Schloegel LM, Marano N, Burgiel S, **Daszak P* (Corresponding Author)**. (2009). Reducing the risks of the wildlife trade. *Science* 324:594-595.
4. Epstein J H, Quan PL, Briese T, Street C, Jabado O, Conlan S, Khan SA, Verdugo D, Hossain MJ, Hutchison SK, Egholm M, Luby SP, **Daszak P* (Co-corresponding Author)**, Lipkin WI. (2010). Identification of GBV-D, a Novel GB-like Flavivirus from Old World Frugivorous Bats (*Pteropus giganteus*) in Bangladesh. PLoS Pathogens 6 (7): e1000972.
5. Homaira N, Rahman M, Hossain MJ, Epstein JH, Sultana R, Khan MSU, Podder G, Nahar K, Ahmed B, Gurley ES, **Daszak P**, Lipkin WI, Rollin PE, Comer JA, Ksiazek TG & Luby SP. (2010). Nipah virus outbreak with person-to-person transmission in Thakurgaon, Bangladesh 2007. *Epidemiol & Infection* 138: 1630-1636.
6. Olival KJ, Islam A, Yu M, Anthony SJ, Epstein JH, Khan SA, Khan SU, Crameri G, Wang L-F, Lipkin WI, Luby SP, **Daszak P** (2013). Filovirus antibodies in fruit bats, Bangladesh. *Emerg. Infect. Dis.* 19: 270-273.
7. Sazzad HMS, Hossain MJ, Gurley ES, Ameen KMH, Parveen S, Islam MS, Faruque LI, Podder G, Banu SS, Lo MK, Rollin PE, Rota PA, **Daszak P**, Rahman M, Luby SP. (2013). Nipah virus infection outbreak with nosocomial and corpse-to-human transmission, Bangladesh. *Emerg. Infect. Dis.* 19: 210-217.

D. Research Support

Ongoing Research Support

NSF Daszak (PI) 07/01/10-06/30/15
EcoHealthNet - a Research Coordination Network
Funding for student exchange and workshops to fuse veterinary science, ecology and human medical sciences
Role: PI

5R01GM100471 Perrings (PI) 09/15/11-06/30/15
NIGMS
Modeling Anthropogenic Effects in the Spread of Infectious Disease
Role: Co-Investigator

1R56TW009502 Daszak (PI) 09/17/12-08/31/14
NIH Fogarty International Center
Comparative Spillover Dynamics of Avian Influenza in Endemic Countries
Role: PI

USAID EPT PREDICT Daszak (PI) 10/01/09 – 09/30/14
Modeling hotspots for disease emergence and conducting surveillance in wildlife in hotspots for new emerging zoonoses
Role: PI on Subcontract

2 R01TW005869 Daszak (PI) 09/01/08 – 08/31/13
NIH Ecology of Infectious Diseases (Fogarty International Center)
The Ecology, Emergence and Pandemic Potential of Nipah virus in Bangladesh
To conduct mathematical modeling and fieldwork to understand the dynamics of Nipah virus in Bangladesh
Role: PI

NSF DEB-1257513 Daszak (PI) 08/15/12-07/31/13
US-China Ecology and Evolution of Infectious Diseases Collaborative Workshop; Kunming, China - October, 2012
Role: PI

1 R01AI079231 Daszak (PI) 09/18/08 – 08/31/13
NIAID Non-Biodefense Emerging Infectious Diseases
Risk of viral emergence from bats.
To model hotspots for bat viral diversity, identify & characterize new bat viruses & understand their pathology
Role: PI

HDTRA1-13-C-0029 Preston(PI) 01/11/13-01/10/14
Office of Naval Research, Defense Threat Reduction Agency
Global Rapid Identification Tool (GRIT) for Undiagnosed Emerging Infectious Diseases (EID) Events
Role: Co-Investigator

Completed Research Support

NSF BCS 0826779 Daszak (PI) 10/01/08 – 03/31/12
NSF Human and Social Dynamics
AOC - HSD – Collaborative Research: Human-related factors affecting emerging infectious diseases
To analyze how socio-economic and environmental drivers predict risk of EIDs
Role: PI on lead proposal

R01TW005869 - supplemental Daszak (PI) 09/01/08 – 08/31/11
NIH EID (Fogarty International Center)
Supplemental funding: Predicting the risk of global H5N1 spread
This project will involve mathematical modeling and fieldwork in Bangladesh and China to understand risk of H5N1 spread.
Role: PI

NSF EF-062239 Kilpatrick (PI) 09/01/06 - 08/30/11
NSF/NIH: Ecology & Evolution of Infectious Diseases
Predicting spatial variation in West Nile virus transmission
Study interaction among WNV vector, reservoir host populations across an urban-to-rural gradient.
Role: Co-PI

R01 TW05869 Daszak (PI) 08/01/02 - 05/31/07
NIH/Fogarty International Center
Anthropogenic change & emerging zoonotic paramyxoviruses
To identify the cause of emergence of Nipah and Hendra viruses in Malaysia and Australia.
Role: PI

HSD 0525216 Daszak (PI) 10/15/05 - 10/14/06
National Science Foundation: Human and Social Dynamics
Collaborative Research: Socio-Economic and Environmental Drivers of Emerging Diseases
To analyze patterns of disease emergence globally and produce a broad risk assessment.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Zhengli Shi	POSITION TITLE Senior scientist
eRA COMMONS USER NAME (credential, e.g., agency login) (b) (6)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Department of Biology, Wuhan University, China	B.S.	1987	GENETICS
Wuhan Institute of Virology, Chinese Academy of Sciences, China	M.S.	1990	VIROLOGY
University Montpellier II, Montpellier, France	Ph.D.	2000	VIROLOGY

A. Personal Statement

The focus of this project is to understand the risk of coronavirus spillover from bats to people in China, using ecological analyses, fieldwork, receptor binding assays, and modeling approaches. I have worked in lab-based virology for 23 years, specializing in SARS-CoV and SARS-like CoVs since 2002. This includes the discovery of a wide-array of SARS-like coronaviruses in mainland China, including two isolates able to bind to the ACE2 receptor. My lab has established several bat primary cell lines and immortalized cell lines, capacity for pseudovirus generation and SARS-specific binding assays and we have expertise in every laboratory technique in this proposal. I have collaborated with the PI for over 10 years, and have spent time in laboratories in the USA and Europe. My lab will be responsible for diagnosis, genomics and isolation of coronavirus from wild and domestic animals in Southern China and for analyzing their receptor binding domains.

B. Positions and Honors.

Positions and Employment

1990-1993	Research assistant, Wuhan Institute of Virology, Chinese Academy of Sciences, China
1993-1995	Research scientist, Wuhan Institute of Virology, Chinese Academy of Sciences, China
2000-	Senior Scientist, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China

Other Experience and Professional Memberships

2008-	Member, American Society of Microbiology
2001-	Member, Chinese Society of Microbilogy
2001-	Member, Chinese Society of Biochemistry and Molecular Biology
2004-	Editor board, Chinese Journal of Virology
2004-2009	Editor board, Virologica Sinica
2010-	Associate Editor, Virologica Sinica

Honors

1996	Chinese Government Graduate Scholarship, the Ministry of Education, PR China.
2003	Natural Science Award (the second rank) of Hubei province, China.
2004	Outstanding supervisor of graduate student of Hubei province, China.
2005	Visitor scholarship from the Chinese Academy of Sciences.
2006	Outstanding scientist of the Chinese Academy of Sciences.

C. Selected peer-reviewed publications (Selected from 82 peer-reviewed)

Most relevant to the current application

1. Li, W., Shi Z., Yu M., Ren W., Smith C., Epstein H. J., Zhang S., Wang H., Crameri G., Hu Z., Zhang H., Zhang J., Mceachern J., Field H., Daszak P., Eaton T.B. and Wang L. F. (2005). Bats are natural reservoirs of SARS-like coronaviruses. Science, 310(5748), 676-679.
2. Hon, C. C., Lam, T. Y., Shi, Z., Drummond, A. J., Yip, C. W., Zeng, F., Lam, P. Y. and Leung, F. C.. (2008). Evidence of the recombinant origin of a bat severe acute respiratory syndrome (SARS)-like coronavirus and its implications on the direct ancestor of SARS coronavirus. Journal of Virology, 82(4), 1819-1826.
3. Yuan, J., Hon,C. C., Li, Y., Wang, D., Xu, G., Zhang, H., Zhou, P., Poon, L. M., Lam, T. T. Leung, F. C. and Shi, Z. (2010). Intra-species Diversity of SARS-Like Coronaviruses (CoVs) in Rhinolophus sinicus and Its Implications on the Origin of SARS-CoVs in human. Journal of General Virology, 91(4),1058-1062.
4. Hou, Y., Peng, C., Yu, M., Li,Y., Han, Z., Wang, L-F., Li, F., Shi, Z. (2010). Bat Angiotensin Converting Enzyme-2 Displays Different Receptor Activity to Severe Acute Respiratory Syndrome Coronavirus Entry. Archives of Virology, 155(10), 1563-1569.
5. Wang, J., Wang, L-F. and Shi, Z. (2008). Construction of a non-infectious SARS coronavirus replicon for application in drug screening and analysis of viral protein function. Biochemical and Biophysical Research Communications, 374(1),138-142.

<u>Additional recent publications of importance to the field (in chronological order)</u>

1. Ren, W., Li, W., Yu, M., Hao, P., Zhang, Y., Zhou, P., Zhang, S., Zhao, G., Zhong, Y., Wang, S., Wang, L. F. and Shi, Z. (2006). Full genome sequences of two SARS-like coronaviruses in horseshoe bats and genetic variation analysis. Journal of General Virology, 87(11), 3355-3359.
2. Li,Y., Wang, J., Hickey, A. C., Zhang, Y., Li, Y., Wu, Y., Zhang, H., Yuan, J., Han, Z., McEachern, J., Broder, C. C., Wang, L. F. and Shi, Z. (2008). Potential nipah virus infection in Chinese bats. Emerging Infectious Diseases, 14(12),1974-1976.
3. Ren, W., Qu, X., Li, W., Han, Z., Yu, M., Zhang, S., Wang, L. F., Deng, H., Shi, Z. (2008). Difference in receptor usage between SARS coronavirus and SARS-like coronavirus of bat origin. Journal of Virology, 82(4), 1899–1907.
4. Zhou, P., Han, Z., Wang, L.F. and Shi, Z. (2009). Immunogenicity difference between the SARS coronavirus and the bat SARS-like coronavirus spike (S) proteins. Biochemical and Biophysical Research Communications, 387(2), 326-329.
5. Li, Y., Ge X., Hon C. C., Zhang H., Zhou P., Zhang Y., Wang L. F. and Shi Z. (2010). Prevalence and Genetic Diversity of Adeno-Associated Viruses in Bats, China. Journal of General Virology, 91(10), 2601-2609.
6. Zhang Y., Zhang H., Dong X., Yuan J., Zhang H., Yang X., Zhou Peng., Ge X., Li Y., Wang L-F, and Shi Z (2010). Hantavirus Outbreak Associated with Laboratory Rats in Yunnan, China. Infection, Genetics and Evolution, 10(5): 638–644.
7. Li, Y., Ge X., Zhang H., Zhou P., Zhu Y., Zhang Y., Yuan J., Wang L-F., Shi Z. (2010). Host Range, Prevalence and Genetic Diversity of Adenoviruses in Bats. Journal of Virology, 84(8), 3889–3897.
8. Yu, M., Tachedjian, M., Crameri, G., Shi, Z. and Wang, L.F. (2010). Identification of key amino acid residues required for horseshoe bat angiotensin-I converting enzyme 2 to function as a receptor for severe acute respiratory syndrome coronavirus. Journal General Virology, 91(7), 1708-1712.
9. Ge, X., Li, Y., Yang, X., Zhang, H., Zhou, P., Zhang, Y. & Shi, Z. (2012). Metagenomic analysis of viruses from bat fecal samples reveals many novel viruses in insectivorous bats in china. Journal of Virology, 86, 4620-4630.
10. Zhou, P., Li, H., Wang, H., Wang, L. F., Shi, Z. (2012). Bat severe acute respiratory syndrome-like coronavirus ORF3b homologues display different interferon antagonist activities. Journal General Virology, 93, 275-281.

D. Research Support

<u>Ongoing Research Support</u>

30970137 National Natural Science Foundation of China Shi (PI) 01/01/2010-12/31/2012
Metagenomic analysis of bat intestinal viruses
Role: PI

2011CB504700 National Basic Research program of China Shi (PI) 01/01/2011-12/31/2015

Mechanism of interspecies transmission of zoonotic viruses
Role: PI

81290341 National Natural Science Foundation of China Shi (PI) 01/01/2013-12/31/2017
Genetic diversity, identification and pathogenesis of bat viruses
Role: PI

Completed Research Support

2005CB523004 National Basic Research program of China Shi (PI) 01/01/2006-12/31/2010
Interspecies transmission mechanism of zoonotic viruses
Role: PI

2009ZX10004-109 Key project of infectious diseases Shi (PI) 01/01/2009-12/31/2010
Rapid and high throughput diagnostic methods for emerging infectious viral pathogens
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Zhang, Shuyi	Dean
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted] (b) (6)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Northeast Normal University, China	B.Sc	07/87	Biology
University of Paris XIII, France	D.E.A.	10/90	Ethology
University Pierre & Marie Curie, France	Ph.D.	12/94	Primatology, Tropical Ecology

A. Personal Statement

The goal of the current proposal is to work on the ecology and evolutionary biology of a coronaviruses from wildlife, with special emphasis on China. My background is ideally suited to this work because I am originally trained as a wildlife biologist, but have been working on the ecology and evolutionary biology of zoonoses in wildlife for the past decade. My career as a wildlife biologist began with a Ph.D in France on the behavioral ecology of capuchin monkeys (*Cebus apella*) in primary forest of French Guiana. In 1995, I returned to China, working on golden monkeys (*Rinopithecus*) at the Institute of Zoology, Chinese Academy of Sciences. At the same time, I began to work on the behavioral ecology of bats and rapidly became the leading bat researcher in China, building a large, well-funded group working on the phylogeny, genetics and ecology of bats. In 2003, during the early outbreak of SARS epidemic, I was one of the few Chinese researchers hypothesizing that SARS must have originated from wild birds or mammals, and I became closely involved in the work of the WHO veterinary team investigating potential wildlife reservoirs for SARS. I continued this work with Drs Zhengli Shi, Peter Daszak and Jon Epstein after the WHO team had left, and discovered that bats are the natural reservoir of SARS-like CoVs. After we published our results in *Science* in 2005, I continued to work on bat CoVs, bat genetics, molecular biology and immunology. I have worked actively with the EcoHealth Alliance and with the Wuhan Institute of Virology, and am involved in most of the preliminary data that is listed in the current application. I also act as the main, on-the-ground contact for EcoHealth Alliance research in China, and am Country Coordinator for the USAID-EPT PREDICT program. During the past decade, I have demonstrated my capacity to provide access to some of the most sensitive fieldwork sites in China and collaborate with US institutions in this work. This includes: collaborative work at Xinghai Lake, where we successfully isolated the first H5N1 from wild birds; work on hunter-trader cohorts in the wet markets of Guangzhou, where we are collaborating with EcoHealth Alliance to identify novel pathogens spilling over from wildlife to people; and the work we conducted on bat SARS-like CoVs in 20034, which involved the export of samples from wildlife into foreign collaborators labs for sequencing and pathogen discovery. In my current capacity as Dean of a 3-institute collaboration at ECNU, I have unique capability to mobilize resources, and work within my large network of collaborators to facilitate the current project.

B. Positions and Honors

Positions and Employment

2011-	Dean, Institutes for Advanced Interdisciplinary Research, East China Normal University, China
2010-	Country Coordinator, USAID-EPT PREDICT
2006-2008	Professor, School of Life Science, East China Normal University, China
1997-2006	Research Professor, Institute of Zoology, Chinese Academy of Sciences, China
1995-1997	Associate Research Professor, Institute of Zoology, Chinese Academy of Sciences, China
1995	Assistant Research Professor, Institute of Zoology, Chinese Academy of Sciences, China

Other Experience and Professional Memberships

1997-	Chairman of China's Primate Specialist Group, Species Survival Commission, World

	Conservation Union (IUCN-SSC)
1999-	Secretary General of Bat Specialists Group of China's Mammalogical Society
2000-	Member of Chinese National Committee for International Union of Biological Sciences

Honors

1989	Fellowship from the China's Education Ministry for students abroad
1991	Fellowship from the French Government for Chinese students
1995	Research grant under the "100 Talent Programme" sponsored by the Chinese Academy of Sciences
1998	Allowance of the State Department for research and technology
1999	Research grant under the "Young Scientist" sponsored by the Chinese Academy of Sciences
2000	“Excellent Young Researcher Grant” of the National Natural Science Foundation of China
2001	“Young Scientist” award of the Chinese Academy of Sciences
2006	Nation Award (class II) for Science and Technology

C. Peer-reviewed publications (selected from over 180 peer-reviewed publications)

Most relevant to the current application

1. He, J.F., Peng, G.W., Min, J., Yu, D.W., Liang, W.J., Zhang, S.Y., Xu, R.H., Zheng, H.Y., Wu, X.W., Xu, J., Fang, L., Zhang, X., Li, H., Yan, X.G., Lu, J.H., Hu, Z.H., Huang, J.C., Wan, Z.Y., Lin, J.Y., Song, H.D., Wang, S.Y., Zhou, X.J., Zhang, G.W., Guo, B.W., Zheng, H.J., Zhang, X.L., Zheng, K., Wang, B.F., Fu, G., Hou, J.L., Wang, X.N., Chen, S.J., Hao, P., Tang, H., Ren, S.X., Zhong, Y., Guo, Z.M., Liu, Q., Miao, Y.G., Kong, X.Y., He, W.Z., Li, Y.X., Chen, Z., Wu, C-I, Zhao, G.P., Chiu, R.W.K., Chim, S.S.C., Tong, Y.K., Chan, P.K.S., Tan, J.S., Lo, Y.M.D. (2004). Molecular evolution of the SARS-coronavirus during the course of the SARS epidemic in China. Science, 303, 1666-1669.
2. Li, W.D., Shi, Z.L., Yu, M., Ren, W.Z., Smith, C., Epstein, J., Wang, H.Z., Crameri, G., Hu, Z.H., Zhang, H.J., Zhang, J.H., McEachern, J., Field, H., Daszak, P., Eaton, B.T., Zhang, S.Y., Wang, L.F. (2005). Bats are natural reservoirs of SARS-like coronaviruses. Science, 310, 676-679.
3. Tang, X.C., Zhang, J.X., Zhang, S.Y., Wang, P., Fan, X.H., Li, L.F., Li, G., Dong, B.Q., Liu, W., Cheung, C.L., Xu, K.M., Song, W.J., Vijaykrishna, D., Poon, L.L.M., Peiris, J.S.M., Smith, G.J.D., Chen, H., Guan, Y. (2006). Prevalence and genetic diversity of coronaviruses in bats from China. Journal of Virology, 80, 7481-7490.
4. Ren, W.Z., Qu, X.X., Li, W.D., Han, Z.G., Yu, M., Zhou, P., Zhang, S.Y., Wang, L.F., Deng, H.K., Shi, Z.L. (2008). Difference in receptor usage between SARS coronavirus and SARS-like coronavirus of bat origin. Journal of Virology, 82, 1899-1907.
5. Tang, X.C., Li, G., Vasilakis, N., Zhang, Y., Shi, Z.L., Zhong, Y., Wang, L.F., Zhang, S.Y. (2009). Differential stepwise evolution of SARS Coronavirus functional proteins in different host species. BMC Evolutionary Biology 9, 52, doi:10.1186/1471-2148-9-52.

Additional recent publications of importance to the field

1. Wang, L.F., Shi, Z.L., Zhang, S.Y., Field, H., Daszak, P., Eaton, B.T. (2006). Review of Bats and SARS. Emerging and Infectious Disease, 12, 1834 -1840.
2. Li, G., Jones, G., Rossiter, S., Chen, S.F., Parsons, S., Zhang, S.Y. (2006). Phylogenetics of small horseshoe bats from East Asia based on mitochondrial DNA sequence variation. Journal of Mammalogy, 87, 1234-1240.
3. Ren, W.Z., Li, W.D., Yu, M., Hao, P., Zhou, P., Zhang, S.Y., Zhao, G.P., Zhong, Y., Wang, S.Y., Wang, L.F., Shi, Z.L. (2006). Full-length genome sequences of two SARS-like coronaviruses in 4 horseshoe bats and genetic variation analysis. Journal of General Virology, 87, 3355–3359.
4. Cui, J., Han, N.J., Streicker, D., Li, G., Tang, X.C., Shi, Z.L., Hu, Z.H., Zhao, G.P., Guan, Y., Wang, L.F., Field, H., Jones, G., Daszak, P., Zhang, S.Y. (2007). Evolutionary relationships between bat coronaviruses and their hosts. Emerging and Infectious Disease, 13, 1526-1532.
5. Rossiter, S.J., Benda, P., Dietz, C., Zhang, S.Y., Jones, G. (2007). Rangewide phylogeography in the greater horseshoe bat inferred from microsatellites: implications for population history, taxonomy and conservation. Molecular Ecology, 16, 4699-4714.
6. Cui, J., Counor, D., Shen, D., Sun, G.Y., Deubel, V., Zhang, S.Y. (2008). Detection of Japanese

encephalitis virus antibodies in bats, Southern China. American Journal of Tropical Medicine and Hygiene, 78, 1007-1011.

7. Zhang, J.S., Jones, G., Zhang, L.B., Zhu, G.J., Zhang, S.Y. (2010). Recent surveys of bats (*Mammalia: Chiroptera*) from China II. Pteropodidae. Acta Chiropterologica, 12, 103-116.
8. Liu, Y., Cotton, J.A., Shen, B., Han, X.Q., Rossiter, S.J., Zhang, S.Y. (2010). Convergent sequence evolution between echolocating bats and dolphins. Current Biology, 20, R53-54.
9. Zhang, L.B., Parson, S., Daszak, P., Wei, L., Zhu, G.J., Zhang, S.Y. (2010). Variation in the abundance of ectoparasite mites in relation to the reproduction status, age, sex and size of flat-headed bats. Journal of Mammalogy, 91, 136–143.
10. Shen, B., Han, X.Q., Jones, G., Rossiter, S.J., Zhang, S.Y. (2013). Adaptive evolution of Myo6 Gene in Old World Fruit Bats (Family: Pteropodidae). PLOS ONE, 8(4), doi: 10.1371/journal.pone.0062307

D. Research Support

Ongoing Research Support

[redacted] (b) (4) Morse (PI) 10/01/09-09/30/14
PREDICT-Wildlife SMART Surveillance/PREDICT Project to pre-empt at the earlier stages possible, zoonotic diseases that impose significant threat to public health.
Role: Collaborator

[redacted] (b) (4) Zhang (PI) 01/01/11-12/30/13
[redacted] (b) (4): Surveillance Emerging Infectious Diseases This project is to conduct surveillance in wildlife in hotspots for new emerging zoonoses.
Role: PI

[redacted] (b) (4) Zhang (PI) 01/01/11-12/30/13
[redacted] (b) (4): Study of the Evolution of SARS Coronavirus This project is to study the evolutionary relationships between bat coronaviruses and their hosts.
Role: PI

Completed Research Support

[redacted] (b) (4) Zhang (PI) 01/01/09-12/30/12
[redacted] (b) (4) [redacted]: Research on biological characteristics of Bats.
Role: PI

[redacted] (b) (4) Zhang (PI) 01/01/10-12/30/12
Changjiang Scholars and Innovative Research Team in University in China: Studying and Monitoring Wildlife and Zoonosis in Eastern China This project is to identify new viruses from wildlife in Eastern China, and to examine the pathogenicity and infectiousness for these novel pathogens.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Ke, ChangWen	POSITION TITLE Director, Institute of Pathogenic Microbiology Guangdong Center for Disease Control and Prevention
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
West China Medical University	M.P.H.	1984	Public Health
West China Medical University	B.S.	1989	Medicine
Sun Yensen University	M.D.	2001	Biochemistry and Molecular Biology

A. Personal Statement.

I have worked in public health and infectious disease research for more than 10 years. As Director of the Institute of Pathogenic Microbiology at Guangdong CDC I have been involved in the study and control of several emerging zoonitic infections, including SARS CoV and most recently, H7N9 avian influenza. Our work under the Guangdong Department of Health and with several Chinese universities and international collaborators has established several syndromic disease surveillance programs and collaborative infectious disease research programs including Chikungunya, enterovirus 71, Avian influenza H7N9 and H5N1, and SARS CoV. Most recently, through partnership with the USAID PREDICT program, we have augmented our lab's ability to identify zoonotic agents in people highly exposed to wildlife such as those working in live animal markets. This ongoing surveillance program has led to the identification of people who have been exposed to animal pathogens, including SARS CoV, and supports the initiative to extend this type of surveillance to other provinces in China. I believe that there is strong evidence that spillover of animal pathogens to people is occurring in China and MERS CoV in the Middle East shows that we should pay more attention to bat coronaviruses. Given the technical expertise and capacity for disease detection at the microbiology lab at Guangdong CDC, I have high confidence that we will be able to contribute to our understanding of coronavirus circulation in human populations and to determine the risk of new CoVs emerging in China.

B. Positions and Honors

Positions and Employment

1989-2000	Doctor in Charge, Health & Epidemic Prevention Station of Guangdong Province
1994-1996	Participant, Department II of Virology, National Institute of Infectious Diseases, Japan
2003-2004	Visiting researcher, Virology Department, National Institute of Infectious Diseases,Japan
2004-	Director, Institute of Microbiology Center for Disease prevention and Control, Guangdong province, China

Other Experience and Professional Memberships

2004-	Member of National expert committee of Influenza
2006-	Member of National Biosafety expert Committee

C. Selected Peer-reviewed Publications

Most relevant to the current application

1. Mo, H., Zeng, G., Ren, X., Li, H., Ke, C.W., Tan, Y., Cai, C., Lai, K., Chen, R., Chan-Yeung, M., Zhong, N. (2006). Longitudinal profile of antibodies against SARS-coronavirus in SARS patients and their clinical significance. Respirology. Jan; 11(1):49-53.
2. Qiaoli, Z.*,Jianfeng, H., De, W., Zijun, W., Xinguang, Z., Haojie, Z., Fan, D., Zhiquan, L., Shiwen, W., Zhenyu, H., Yonghui, Z., Ke, C.W., Yuan D., Liang W., Li D., Chen, P. (2012). Maiden Outbreak of Chikungunya in Dongguan City, Guangdong Province, China: Epidemiological Characteristics. PLOS ONE, 7(8):1-8
3. Wu, D., Zheng H., Li, H., Monagin, C., Guo, X., Liu, L., Zeng, H., Fang, L., Mo, Y., Zhou, H., Zhang, H., Kou, J., Long, C., Hiromu, Y., & Ke, C.W. (2012). Phylogenetic and molecular characterization of Coxsackievirus A24 variant isolates from a 2010acute hemorrhagic conjunctivitis outbreak in Guangdong, China Virology Journal, 9.41: 1-9
4. Guan, D., van der Sanden, S., Zeng, H., Li, W., Zheng, H., Ma, C., Su, J., Liu, Z., Guo, X., Zhang, X., Liu, L., Koopmans, M., Ke, C.W.* (2012) Population Dynamic and Genetic Diversity of C4 Strains of Human Enterovirus 71 in Mainland China, 1998-2010. PLOS ONE, 7(9):1-8
5. Yang, F., He, J.*, Zhong, H., Ke, C.W., Zhang, X., Hong, T., Ni, H., Lin, J. (2012). Temporal Trends of Influenza A (H1N1) Virus Seroprevalence following 2009 Pandemic Wave in Guangdong, China: Three Cross-Sectional Serology Surveys. PLOS ONE, 7(6):1-8

Additional recent publications of importance to the field (in chronological order)

1. Ke, C.W., Li T.C., Takeda, N. (2005). Positively Charged Amino Acid Residues of VP1 Capsid Protein of Human Polyomavirus BK Influence on the Formation of Virus-like Particles Generated by Recombinant Baculoviruses. Virologica Sinica, 21(1)20-23
2. Ke C.W., Zheng, K., Zhang, X., Zhou H.Q., Duan J.H., Lin L.F. (2005). Detection of Dengue virus by real-time polymerase chain reaction with TaqMan MGB probe. Chinese J Zoonosis, 21(8)716-720
3. Yan, J., Ke, C.W., Zheng, H., et al. (2006). Rapid diagnosis and Identification of Human Enteroviruses by sequencing VP4 gene. Chinese Journal of Vaccines and Immunization. 12(6)469-471
4. Zheng, H.Y., Liu L., Guo, X., Ke, C.W. (2006). A Comparative Study of Three IgM ELISA Kits for Measles Detection. Journal of Tropical Medicine, (08) 897-899
5. Ke, C.W., Deng, F. (2007). Surveillance system based on hospital and laboratory network to discover emerging viral diseases Journal Pathogen Biology, 2(1): 75-76
6. Ke, C.W., Zou, L.R., Yan, J. (2007). Control strategy for emerging Zoonosis. Chinese J Zoonosis, 23(1)92-93.
7. Li, B., Tan, H., Wang, D., et al. (2010). Phenotypic and genotypic characterization of vibrio Cholera O139 of clinical and aquatic isolation in China. Curr. Microbiol.
8. Ding, X., Jiang, L., Ke, C.W. et al. (2010). Amino acid sequence analysis and identification of mutations under positive selection in Hemagglutinin of 2009 influenza A (H1N1) isolates. Virus Genes, 41:329-340
9. Sun, L.M., Zheng, H.Y., Zheng, X.Z. et al. (2011). An enterovirus 71 epidemic in Guangdong province of China, 2008: Epidemiological, Clinical, and Virogenic manifestations. Jpn. J. Ifect. Dis., 64:13-18
10. Su, S., Ning, Z.Y., Zhu, W.J., Jiao, P.R., Ke, C.W., Qi, W.B., Huang, Z., Tian, J., Cao, L., Tan, L.K., Shao, Z.W., Liang, H.B., Huang, W.M., Liao, M., Li, S.J., Zhang, G.H. (2013). Lack of evidence of avian-to-human transmission of avian influenza A (H5N1) virus among veterinarians, Guangdong, China, 2012. Journal of Clinical Virology. 56(4), 365-366.

D. Research Support

Ongoing Research Support

[redacted] (b) (4) 2012-2015

National Major Projects of Major Infectious Disease Control and Prevention: the Ministry of Science and Technology of the People's Republic of China

Completed Research Support

China–U.S. 2009-2012
Collaborative Program on Emerging and Re-Emerging Infectious Diseases
Enhanced surveillance on Salmonella in Guangdong province.

30972591
National Natural Science Foundation of China 2010-2011
Epidemiology and molecular mechanism of virulence mutation of dengue viruses in Guangdong

World Bank 2005
Establish Laboratory Network for Emergency Response and Surveillance of Infectious Diseases in Guangdong Province and Training.

WHO 07.03.01.AW.01.
Epidemiological study on Transmission on Influenza A Virus from Animals to Human

WHO grant: 07.02.01.AW.01.
Surveillance on emerging and reemerging infectious diseases pathogen in Guangdong Province

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Jonathan H. Epstein	Associate Vice President & Asia Regional Coordinator
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted] (b) (6)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Brandeis University, MA	BA	1996	Biology
Tufts University, Sch. Vet. Med., Boston, MA	DVM	2002	Wildlife Med., Intl. Med.
Tufts University, Sch. Vet. Med., Boston, MA	MPH	2002	Epidemiology
Tufts University, Sch. Vet. Med., Boston, MA	Cert Intl Med	2002	Zoonotic Diseases

A. Personal Statement

The goal of the proposed research is to investigate the ecology, evolutionary history and transmission dynamics of mammalian coronaviruses at the human-animal interface. Specifically, we will conduct field studies in China to obtain high quality samples from bats and other mammals found in wet market systems and identify and characterize known and novel coronaviruses. We will analyze the patterns of coronavirus transmission among bats and other wildlife, and the risk of spillover to humans. This research will address fundamentally important issues about the diversity of coronaviruses in mammalian hosts and the risk of inter-species transmission and emergence in human populations. My research has focused on the epidemiology and ecology of emerging zoonotic viruses carried by bats (Nipah virus, Ebolavirus, and SARS CoV), and other wildlife, and the drivers that lead to emergence. My work on SARS CoV ecology, in collaboration with co-investigators Daszak (PI), Zhang and Shi led to the discovery of several SARS-Like coronaviruses in bats, which appear to be ancestral to SARS-CoV and most recently which utilize the same ACE2 receptor as SARS CoV, suggesting direct spillover to humans is possible. Recently, I led a field team in Saudi Arabia in collaboration with the KSA Ministry of Health, to identify the animal origins of the newly discovered **MERS CoV**. I continue to be involved in this ongoing investigation along with co-investigators Daszak and Olival. I have also conducted pathogen discovery work in bats, utilizing next generation sequencing technologies, which led to the discovery of a novel flavivirus related to Hepatitis C virus (GBV D). This team brings a high level of expertise in disease ecology, epidemiology, and pathogen discovery, and includes China's leading experts on wildlife zoonoses in partnership with key provincial CDCs. Our team has maintained a highly productive collaboration under several NIH and non-federally funded research projects, generating peer-reviewed papers in high impact journals (including *Science, PNAS,* and *PLoS Pathogens*). We have proven through previous work that we can manage logistically challenging projects involving people, wildlife, and animals in the wet markets in China, which gives this proposal a high likelihood of success. Under several federal awards, I have successfully managed the field and molecular investigations of zoonotic viruses in bats in several countries including Saudi Arabia, China, India, Malaysia, Thailand, and Bangladesh, all of which have logistical and political challenges. Using the bat and human samples we have already collected; and new animal samples we propose to collect; we will have the resources available to achieve the aims of this proposal.

B. Positions and Honors

Positions and Employment

1999 Intern, Brisbane South Public Health Unit & DPI Queensland Animal Research Institute, AUS.
2002 Extern, Division of Viral and Rickettsial Diseases, CDC, Atlanta, GA
2002 Veterinary Intern, Small animal emergency and critical care, Ocean State Vet. Spec., RI
2003- Senior Research Scientist, EcoHealth Alliance, New York, NY.
2003- Adjunct Faculty, Ecology, Columbia Univ., NY & Tufts University Sch. of Vet. Med., MA.
2006- Adjunct Faculty, Mailman School of Public Health, Columbia Univ, NY
2007- Adjunct Asst. Clinical Professor, Public Health & Family Med, Tufts Univ School of Medicine, MA

2008- Postdoctoral fellow, Center for Infection and Immunity, Columbia University, NY, Adjunct Associate Professor, Mt. Sinai School of Medicine
2008- Review Editor - *EcoHealth*
2009- Associate Vice President, Conservation Medicine Program EcoHealth Alliance, NY
2009- Executive Director, Consortium for Conservation Medicine, EcoHealth Alliance, NY
2009- Asia Regional Coordinator, USAID EPT (PREDICT)
2011- Admissions committee, Tufts University Masters in Conservation Medicine degree program
2012- Board of Directors, International Association of Ecology and Health; Scientific Advisory Board, Lubee Bat Conservancy

Other Experience and Professional Memberships

1998- Member: American Veterinary Medicinal Association, American Association of Zoo Vets, Wildlife Disease Association, New York Academy of Sciences,
2003- Member, IUCN Veterinary Specialist Group
2004 Invited speaker, WHO, Emerging Zoonotic Diseases Working Group meeting
2004 Member and Health Advisor, IUCN Bat Specialist Group; Advisory committee, Suffolk Country Board of Public Health; International Assoc. Ecology and Health
2006 Member, Delta Omega Public Health Honors Society
2007- Leader, Vertebrate Health Task Force, Smithsonian Institution Geological Earth Observatory Program (SIGEO)
2010- Scientific Committee Member, DIVERISTAS ecoHEALTH cross-cutting network (ICSU-UNESCO)
2008-13. (selected) Invited presentations: University of Malaysia, Sarawak – Emerging zoonoses; **IOM-NAS** Committee on Achieving sustainable global capacity for surveillance and response to emerging infectious diseases; Nipah virus colloquium, University Malaya, Kuala Lumpur, Malaysia, Nipah virus symposium, American Society for Tropical Medicine & Hygiene; International Bat Research Symposium, Prague; American Society of Microbiology, Washington, DC; Australian Animal Health Laboratory (AAHL), Geelong; International Meeting on Emerging Diseases (IMED), Vienna; **IOM** meeting on MERS CoV and H7N9, Washington DC.

Honors

2002 First recipient, Certificate of International Veterinary Medicine, Tufts University Sch. Vet. Med.
2002 Hills award for excellence in veterinary clinical nutrition
2002 Sylvia Mainzer award for outstanding achievement in the field of public health
2004 NIH Loan Repayment Award (competitive award for Nipah virus research)
2006 Inducted into Delta Omega Honor Society for Public Health (Alpha Rho Chapter – 1st alumni inductee; 1st Inaugural Keynote Speaker)
2007 Outstanding Alumnus award, Tufts Cummings School of Veterinary Medicine
2012 Young Alumni Achievement Award, Tufts University (selected from all alumni who graduated in past 10 yrs)

D. Selected peer-reviewed publications (from 45). * indicates corresponding author

Most relevant to the application (in chronological order)

1. Li, W., Shi, Z., Yu, M., Ren, W., Smith, C., Epstein, J.H., Wang, H., Crameri, G., Hu, Z., Zhang, H., Zhang, J., McEachern, J., Field, H., Daszak, P., Eaton, B.T., Zhang, S. & Wang, L-F. (2005). Bats are natural reservoirs of SARS-like coronaviruses. Science 310: 676-679.
2. Epstein, J.H.*, Quan, P.L., Briese, T., Street, C., Jabado, O., Conlan, S., Khan, S.A., Verdugo, D., Hossain, M.J., Hutchison, S.K., Egholm, M., Luby, S.P., Daszak, P., & Lipkin, W.I. (2010). Identification of GBV-D, a Novel GB-like Flavivirus from Old World Frugivorous Bats (Pteropus giganteus) in Bangladesh. PLoS Pathogens 6(7): e1000972. doi:10.1371/journal.ppat.1000972.
3. Anthony, S.J, Ojeda-Flores, R., Rico-Chávez, O., Navarrete-Macias, I., Zambrana-Torrelio, C.M., Rostal, M.K., Epstein, J.H., Tipps, T., Liang, E., Sanchez-Leon, M., Sotomayor-Bonilla, J., Aguirre, A.A., Ávila, R., Medellín, R.A., Goldstein, T., Suzán, G., Daszak, P., Lipkin, W.I. (2013). Coronaviruses in bats from Mexico. J. Gen Virol. Published ahead of print January 30, 2013, doi:10.1099/vir.0.049759-0

4. Wacharapluesadee, S., Sintunawa, C., Kaewpom, T., Khongnomnan, K., Olival, K.J., Epstein, J.H., et al. (2013). Group C betacoronavirus from bat guano 11 fertilizer, Thailand. Emerg Infect Dis. Aug. 12 http://dx.doi.org/10.3201/eid1908.130119
5. Quan, P.L., Firth, C., Conte, J.M., Williams, S.H., Zambrana-Torrelio, C.M., Anthony, S.J., Ellison, J.A., Gilbert, A.T., Kuzmin, I.V., Niezgoda, M., Osinubi, M.O.V., Recuenco, S., Markotter, W., Breiman, R.F., Kalemba, L., Malekani, J., Lindblade, K.A., Rostal, M.K., Ojeda-Flores, R., Suzan, G., D., Lora B., Blau, D.M., Ogunkoya, A.B., Alvarez C., Danilo A., Moran, D., Ngam, S., Akaibe, D., Agwanda, B., Briese, T., Epstein, J.H., Daszak, P., Rupprecht, C.E., Holmes, E.C., & Lipkin, W.I. (2013). Bats are a major natural reservoir for hepaciviruses and pegiviruses. PNAS. doi:10.1073/pnas.1303037110

Additional recent publications of importance to the field (in chronological order)

1. Epstein, J.H.*, Field, H.E., Luby, S., Pulliam, J., & Daszak, P. (2006). Nipah Virus: Impact, Origins, and Causes of Emergence. Current Infectious Disease Reports 8: 59-65.
2. Epstein, J.H.*, Rahman, S.A., Zambriski, J.A., Halpin, K., Meehan, G., Jamaluddin, A.A., Hassan, S.S., Field, H.E., Hyatt, A.D., Daszak, P. & HERG. (2006). Feral cats (Felis catus) as possible vectors for Nipah virus. Emerging Infectious Diseases. 12: 1178-1179.
3. Field, H.E., Wang, L.F., Zhang, S., Daszak, P., Smith, C.S., Epstein, J.H., Shi, Z. (2007). Searching for the natural reservoir of the SARS virus. Preventive Veterinary Medicine. 81(1-3): 216-216 Sp. Issue.
4. Epstein, J.H.*, Prakash, V., Smith, C.S., Daszak, P., McLaughlin, A.B., Meehan, G., Field, H.E., and Cunningham, A.A. (2008). Evidence for Henipavirus infection in Indian Pteropus giganteus (Chiroptera; Pteropodidae) fruit bats. Emerging Infectious Diseases 14(8). 1309-11.
5. Epstein, J.H.*, Olival, K.J., Pulliam, J.R.C., Smith, C., Westrum, J., Hughes, T., et al. (2009). Pteropus vampyrus, a hunted migratory species with a multinational home-range and a need for regional management. Journal of Applied Ecology. 46(5):991-1002.
6. Epstein, J.H.*, Price, J.T. (2009). The Significant but Understudied Impact of Pathogen Transmission from Humans to Animals. Mount Sinai Journal of Medicine 76(5):448-55.
7. Homaira, N., Rahman, M., Hossain, M. J., Epstein, J.H., Sultana, R., Khan, M.S.U., Podder, G., Nahar, K., Gurley, E.S., Daszak, P., Lipkin W.I., Rollin, P.E., Comer, J.A., Ksiazek, T.G., Luby, S.P. (2010). Nipah outbreak with person-to-person transmission in Thakurgaon, Bangladesh, 2007. Epidemiology and Infection. 138: 1630-1636.
8. Sohayati, A., Rahman, Hassan, S.S, Olival, K.J., Mohamed, M., Chang, L-Y., Hassan, L., Suri, A.S., Saad, N.M., Shohaimi, S.A., Mamat, Z.C., Epstein, J.H., Field, H.E., Daszak, P., & HERG. (2010). Genetic characterization of Nipah virus isolated from naturally infected Pteropus vampyrus in Malaysia. Emerging Infectious Diseases.16(12).1990-3.
9. Pulliam, J.R., Epstein, J.H., Dushoff, J., Rahman, S.A., Meehan, G., Bunning, M., HERG, Jamaluddin, A.A., Hyatt, A.D., Field, H.E., Dobson, A.P. & Daszak, P. (2011). Agricultural intensification, priming for persistence, and the emergence of Nipah virus: a lethal bat-borne zoonoses. Journal of the Royal Society, Interface. Doi:10.1098/rsif.2011.0223 (journal's most cited article in 2012)
10. Halpin, K., Hyatt, A.D., Fogarty, R., Middleton, D., Bingham, J., Epstein, J.H., Rahman, S.A., Hughes, T., Smith, C., Field, H.E., Daszak, P., & the Henipavirus Ecology Research Group. (2011). Pteropid Bats are Confirmed as the Reservoir Hosts of Henipaviruses: A Comprehensive Experimental Study of Virus Transmission. Am J Trop Med Hyg. 85:946-951; doi:10.4269/ajtmh.2011.10-0567
11. Sohayati, R., Hassan, L., Sharifah, S.H., Lazarus, K., Zaini, C.M., Epstein, J.H., Naim, N.S., Field, H. E., Arshad, S.S., Aziz, J.A., & Daszak, P. (2011). Evidence for Nipah virus recrudescence and serological patterns of captive Pteropus vampyrus. Epidemiology and Infection. 139, pp 1570-1579 doi:10.1017/S0950268811000550
12. Daszak, P., Zambrana-Torrelio, C., Bogich, T.L., Fernandez, M., Epstein, J.H., Murray, K.A. & Hamilton, H. (2012). Interdisciplinary approaches to understanding disease emergence: The past, present and future drivers of Nipah virus emergence. PNAS doi:10.1073/pnas.1201243109

D. Research Support
Ongoing Research Support
Ongoing Research Support

USAID Daszak (PI) 10/01/09 – 09/30/14
Emerging Pandemic Threats: PREDICT
Modeling hotspots for disease emergence and conducting surveillance in wildlife for new emerging zoonoses.
Role: Asia Regional Coordinator: coordinating field and lab activities in Bangladesh, India, Thailand, Malaysia, Indonesia and China; Surveillance Team and Molecular Diagnostic team member.

2 R01TW005869 Daszak (PI) 09/01/08 – 08/31/14
NIH Ecology of Infectious Diseases (Fogarty International Center)
The Ecology, Emergence and Pandemic Potential of Nipah virus in Bangladesh
To conduct mathematical modeling and fieldwork to understand the dynamics of Nipah virus in Bangladesh
Role: Co-PI

1 R01AI079231 Daszak (PI) 09/18/08 – 08/31/13
NIAID Non-Biodefense Emerging Infectious Diseases
Risk of viral emergence from bats. This project is to model hotspots for viral diversity and emergence in bats, to identify new viruses from bats, and to examine the pathogenicity and infectiousness for these novel pathogens. Role: Co-PI

0955897 NSF Research Coordination Network Daszak (PI) 07/01/10-06/30/15
EcoHealthNet: Environmental Science and Health Research Network
The major goal of this research is to run a series of workshops and student research exchange programs focused on collaborations among the human medical, veterinary, ecological and economic sciences.
Role: Co-PI, Program Director

USFWS, F12AP01117 Epstein (PI).
Development of a Great Ape Health Unit in Sabah, Malaysia 09/13/12 - 09/13/14

USFWS, 4500036150 Epstein (PI) 09/15/12 - 09/14/14
Characterization of Climatic Parameters within Bat Hibernacula, their Influence on Environmental Loads of *Geomyces destructans*, and Implications for the Migration of White-Nose Syndrome in Bats

Completed Research Support

1K08AI067549 Epstein (PI) 07/1/07 – 07/30/11
Understanding the Ecology of Nipah Virus in Bangladesh (NIAID)
Modeling the dynamics of Nipah virus in *Pteropus giganteus* and risk of spillover to humans.
Role: PI (collecting Nipah virus epidemiological data from Bats in Bangladesh)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Olival, Kevin James	POSITION TITLE Senior Research Scientist
eRA COMMONS USER NAME (credential, e.g., agency login) (b) (6)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Colorado State University	B.S.	05/97	Biology
Columbia University	MA	10/03	Conservation Biology
Columbia University	PhD	05/08	Ecology and Evolutionary Biology
American Museum of Nat. History	Postdoctoral	08/09	Molec. Parasitology
NIH Fogarty US Global Health Fellow	Postdoctoral	08/11	Int'l. Emerg. Inf. Dis

A. Personal Statement

The goal of our proposal is to study the ecology, evolution, and spillover potential of bat coronaviruses. Specifically, we will use a combination of fieldwork, mathematical modeling, and phylogenetic and molecular methods to test several hypotheses related to zoonotic spillover risk and the limits to host range for bat coronaviruses. My research experiences are strongly complementary to these aims. I have been conducting research on bat evolution, ecology, population genetics, and viral discovery for the past 11 years. During my dissertation at Columbia University, I used host population genetics and phylogeography to understand the dynamics of Nipah virus in Southeast Asian fruit bats. As a post-doc at AMNH I discovered several novel malaria parasites in bats, and used molecular systematics to understand the co-evolution and origins of non-human *Plasmodium*. I developed new approaches that combine phylogenetic, ecological and species life-history variables to predict viral diversity in bats, and have tested these using data from the literature. As an NIH Global Health Fellow, I expanded our knowledge of Nipah virus ecology in Bangladesh through population genetic analyses of the putative primary reservoir host, *Pteropus giganteus*, led field investigations on role of non-*Pteropus* fruit bats in Nipah virus circulation, and discovered a number of novel bat pathogens in these species. I have led field research projects and training workshops to conduct viral discovery throughout Asia, including Malaysia, Bangladesh, India, Vietnam, Cambodia, Thailand, and the Philippines. This included several expeditions to collect Nipah virus samples in Bangladesh and Malaysia, and a three-week long Ebola Reston investigation of bats in the Philippines. Most recently I have led field teams on two 3-week expeditions in Saudi Arabia to identify the animal reservoir of MERS-CoV; this work is ongoing. My latest research is focused on: 1) global viral discovery in bats; 2) integrating phylogenetic and molecular evolution analyses with ecological information to better understand the risk of bat viral spillover. In summary, for the past decade my research has been focused on the evolution and ecology of bats and their associated pathogens, and my current focus of using phylogenetic and evolutionary approaches model viral spillover risk in bats is highly complementary to the aims in this proposal.

B. Positions and Honors

Positions and Employment

2000-02	Mentor, NSF Undergraduate Mentoring in Environmental Biology (UMEB) for Pacific Islander undergraduates, University of Hawaii
2002-08	Research Collaborator, Consortium for Conservation Medicine, New York
2003-	Member, Henipavirus Ecology Research Group
2003	Lecturer in Disease Ecology, Columbia University Continuing Education course
2003-08	Visiting researcher – bat genetics, Veterinary Research Institute, Malaysia
2005	Visiting researcher – bat genetics, Institute for Ecology and Biological Resources, Vietnam
2005	Visiting researcher – bat genetics, Pasteur Institute, Cambodia
2005	Judge, NY Science and Engineering Fair
2006-07	Mentor, Conservation Genetics High School Internship Program, AMNH, New York
2006-13	Instructor, Columbia University Secondary School Summer Program, New York
2007	Steering Committee, Small Matters: Microbes and Their Role in Conservation, New York
2007	Symposium Organizer, Bat Hunting and Bushmeat, Phuket, Thailand
2009	Symposium Organizer, Bat migration and disease, 1^{st} Int'l Workshop on Bat Migration, Germany
2009	Organizer and Scientific Review Committee, Exploring the Dynamic Relationship Between Health and the Environment, AMNH Spring Symposium, New York
2009-	Review Editor, EcoHealth
2009-	Adjunct Research Faculty, Center for Environmental Sustainability, Columbia University, New York.
2009-	Visiting Research Scientist, American Museum of Natural History, Mammalogy Department.
2010	Mentor and Scientific Review Committee, Student Conference on Conservation Science New York
2010-	Key Personnel and Lead Country Liaison: Thailand, Bangladesh, and Vietnam - USAID PREDICT
2010-	Lead Field Researcher, FAO-EHA investigation of Ebola Reston reservoirs in Philippines
2011-	Steering Committee, NSF RCN grant, South-east Asian Bat Conservation Research Group
2011-	Internship Mentor, NSF RCN grant EcoHealthNet, graduate training in One Health
2013-	EHA Team lead; MERS-CoV animal reservoir investigations with MoH in Saudi Arabia

Honors

1993-97	Colorado State University Distinguished Scholar Award
2003	NSF Graduate Student Fellowship, Honorable Mention
2005-07	Bat Conservation International Student Award and Scholarship
2004-07	US Environmental Protection Agency STAR Fellowship Award
2008	PhD Dissertation with Distinction, Columbia University
2013	Plenary talk on bat virus modeling at 11^{th} Annual ASM Biodefense and EID Research Meeting
2013	Invitation to participate in Institute of Medicine panel on novel Coronavirus

C. Selected Peer-reviewed Publications (Selected from 25 peer-reviewed publications)

Most relevant to the current application

1. Turmelle, A. & Olival, K.J. (2009). Correlates of viral richness in bats (Order Chiroptera). EcoHealth 6(4): 522-39.
2. Rahman, S.A., Hassan, SS, Olival, K.J., Mohamed, M., Chang, L.Y., Hassan, L., Saad, N.M., Shohaimi, S.A., Mamat, Z.C., Naim, M.S., Epstein, J.H., Suri, A.S., Field, H.E., Daszak, P. & HERG. (2010). Characterization of Nipah virus from Naturally Infected *Pteropus vampyrus* Bats, Malaysia. Emerging Infectious Diseases 16(12): 1990-93.
3. Olival, K.J., Epstein, J.H., Wang, L.F., Field, H.E., & Daszak, P. (2012). Are bats unique viral reservoirs? in A. A. Aguirre, R. S. Ostfeld, and P. Daszak, editors. New Directions in Conservation Medicine: Applied Cases of Ecological Health. Oxford University Press, Oxford. pp. 195-212.
4. Levinson, J., Bogich, T.L., Olival, K.J., Epstein, J.H., Johnson, C.K., Karesh, W. & Daszak, P. (2013). Targetting surveillance for zoonotic virus discovery. Emerging Infectious Diseases 19(5): 743-47.
5. [redacted] (b) (4)

Identification of Group C Betacoronavirus from Bat guano fertilizer, Thailand. Emerging Infectious Diseases.

Additional recent publications of importance to the field (in chronological order)

1. Olival, K.J. & Daszak, P. (2005). The ecology of emerging neurotropic viruses. Journal of NeuroVirology 11: 440-45.
2. Pulliam, J.R.C., Field, H.E., Olival, K.J. & HERG. (2005). An alternative explanation of Nipah virus strain variation. Emerging Infectious Diseases 11(12): 1978-1979.
3. Daszak, P., Plowright, R., Epstein, J.H., Pulliam, J.R.C., Rahman, S.A., Field, H.E., Smith, C.S., Olival, K.J., Luby, S., Halpin, K., Hyatt, A.D., & HERG. (2006). The emergence of Nipah and Hendra virus: pathogen dynamics across a wildlife-livestock-human continuum. In: Disease Ecology: Community structure and pathogen dynamics, In Collinge and Ray, ed. Oxford University Press: Oxford. pp. 188-203.
4. Olival, K.J., Stiner, E.O., & Perkins, S.L. (2007). Detection of *Hepatocystis* sp. in Southeast Asian Flying Foxes (Pteropodidae) using Microscopic and Molecular Methods. Journal of Parasitology 93(6): 1538-1540.
5. Epstein, J.H., Olival, K.J., Pulliam, J.R.C., Smith, C.S., Westrum, J., Hughes, T., Dobson, A., Zubaid, A., Rahman, S.A., Basir, M.M., Field, H.E., & Daszak, P. (2009). Management of *Pteropus vampyrus*, a hunted migratory species with a multinational home-range. Journal of Applied Ecology 46(5): 991-1002.
6. Murdock, C., Olival, K.J. & Perkins, S.L. (2010). Feeding preference of snow-melt mosquitoes (Culicidae: Culiseta and Ochelerotatus) show a link between cervid amplifying hosts for Jamestown Canyon Virus (Bunyaviridae: Orthobunyavirus) and humans. Journal of Medical Entomology 47(2): 226-229
7. Smith, C.S., Epstein, J.H., Breed, A., Plowright, R., Olival, K.J., de Jong, C., Daszak, P. & Field, H.E. (2011). Satellite Telemetry and Long-Range Bat Movements. PloS One 6(2): e14696.
8. Bogich, T.L., Olival, K.J., Hosseini, P., Mazet, J., Morse, S., Karesh, W.B., Jones, K.E., Levy, M., Funk, S., Brito, I., Epstein, J.H., Brownstein, J., Joly, D., & Daszak, P. (2012). Using Mathematical Models in a Unified Approach to Predicting the Next Emerging Infectious Disease. New Directions in Conservation Medicine: Applied Cases of Ecological Health. In Aguirre, Ostfeld and Daszak, ed. Oxford University Press. pp. 607-18.
9. Morse, S.F., Olival, K.J., Kosoy, M., Billeter, S.A., Patterson, B.D., Dick, C.W., & Dittmar, K. (2012). Global distribution and genetic diversity of Bartonella in bat flies (Hippoboscoidea, Streblidae, Nycteribiidae). Infection, Genetics and Evolution 12(8): 1717-23.
10. Olival, K.J. (2012). Correlates and evolutionary consequences of population genetic structure in bats. In Gunnell and Simmons, ed. Evolutionary History of Bats: Fossils, Molecules, and Morphology. Cambridge University Press, Cambridge. pp. 267-316.
11. Olival, K.J., Islam, A., Yu, M., Anthony, S.J., Epstein, J.H., Khan, S.A., Khan, S.U., Crameri, G., Wang, L.F., Lipkin, W.I., Luby S.P., & Daszak, P. (2013). Ebolavirus Antibodies in Fruit Bats, Bangladesh. Emerging Infectious Diseases 19(2): 270-273.
12. [redacted] (b) (4)
13. [redacted] (b) (4)

D. Research Support

Ongoing Research Support

NIH 1 R01AI079231 Daszak (PI) 09/18/08 – 08/31/13
NIAID Non-Biodefense Emerging Infectious Diseases. "Risk of viral emergence from bats".
This project is to model hotspots for viral diversity and emergence in bats, to identify new viruses from bats, and to examine the pathogenicity and infectiousness for these novel pathogens.
Role: Key Personnel: lead project implementation, study design, and phylogenetic modeling

USAID EPT PREDICT Daszak (PI) 10/01/09 – 09/30/14
Modeling hotspots for disease emergence and conducting surveillance in wildlife for new emerging zoonoses.
Role: Key Personnel: Modeling disease risk and managing projects in Asian countries

US Geological Survey (USGS) Olival (Co-PI) 06/18/12-06/17/13
"Genetic Approaches to Defining Taxonomic and conservation Units for the Hawaiian Hoary Bat"
Using molecular tools to conserve the endangered Hawaiian Hoary bat.
Role: Co-PI

USFWS 4500036150 Epstein (PI) 09/01/12-12/31/14
Characterization of Climatic Parameters within Bat Hibernacula, their Influence on Environmental Loads of *Geomyces destructans*, and Implications for the Migration of White-Nose Syndrome in Bats.
Role: Co-PI

Completed Research Support

NIH 3R01TW005869-06S1 Daszak (PI) 09/01/09 – 8/31/11
NIH Ecology of Infectious Diseases ARRA supplement to "The Ecology, Emergence and Pandemic Potential of Nipah virus in Bangladesh". Examined the ecology of Nipah virus in Bangladesh; population genetic structure of *P. giganteus*; and the pathogen discovery from a diverse range of bats. Over the course of this award I published >10 papers including 4 in the prestigious journal *Emerging Infectious Diseases*; presented at >20 national and int'l conferences; and media coverage in the *New York Times* Science section.
Role: Fogarty US Global Health Fellow; lead for ARRA reserach

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Parviez R Hosseini	POSITION TITLE Senior Research Fellow
eRA COMMONS USER NAME (b) (6)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Brown University	Sc. B.	12/94	Applied Math – Biology
University of California, Santa Barbara	Ph.D	06/02	Biological Sciences

A. Personal Statement

The aims of the proposed research include disease ecology, evolutionary biology, and understanding the transmission dynamics of coronaviruses among wildlife hosts and their spillover to people. The latter includes analyzing patterns of viral prevalence, building and parameterizing mathematical models of pathogen transmission and evolution, and field research on these dynamics. In my career, I have used my training as theoretical ecologist and my broad experience in mathematics, statistics and ecology to focus on analyzing and explaining the process of disease emergence. I have studied disease ecology, with a strong focus on analytical and computation modeling approaches for the past 9 years. This work has involved leading the modeling component of several major research projects across a wide array of disease systems including *Mycoplasma gallisepticum* in House Finches, Barley and Cereal Yellow Dwarf viruses in California grasslands, Chikungunya virus, Rift Valley fever, and avian influenza. I am now the lead researcher on the modeling component for Influenza and Arbovirus Dynamics at EcoHealth Alliance. My strong interest in the impact of population structure on the emergence of novel pathogens, and my experience in working with computational modeling of emerging diseases give me a perfect background for the current proposed work. I also have considerable experience in working within national and international collaborative groups which will prove invaluable in the current project.

B. Positions and Honors

Positions and Employment

2002-2005	Post-doctoral Associate, Cornell University, Lab of Ornithology, Ithaca, NY
2005-2009	Associate Research Scholar, Princeton University, Dept. of Ecology and Evolutionary Biology. Princeton, NJ
2009-	Senior Research Fellow, EcoHealth Alliance, NY

Professional Activities:

2003 – 2005	Participant, Seasonality and the Population Dynamics of Infectious Diseases, NCEAS, Santa Barbara, CA
2004	Invited Speaker, Ecology of Infectious Disease Meeting, Emory University, Atlanta
2004	Invited Speaker, Dept. of Zoology, Oregon State University
2006	Invited Speaker, Dept. of Biology, EEOB Seminar Series, University of North Carolina
2007	Invited Speaker, Dept. of Ecology and Evolutionary Biology, University of Tennessee
2008	Invited Speaker, Dept. of Zoology, Oxford University, UK

2008	Invited Speaker, Dept. of Biology, Stanford University, CA
2009 – present	Member of modeling team, USAID-EPT PREDICT
2010 – present	Review Editor, EcoHealth

Selected Honors:

2003	NSF RTG/GRT Fellowship on Spatial ecology
2004	Invited to speak at EEID in 2004 and 2011
2004	Member NCEAS group on Recovery plans and de-listing
2005	Member NCEAS group on designing ecological protected areas research
2005	Member NCEAS group on complex population dynamics
2007	*PNAS* 2007 paper cited by Faculty of 1000 Biology as "Must Read"
2007	*PNAS* paper listed as Science Editor's choice, 6th April 2007

C. Peer-reviewed publications

Most relevant to the current application

1. Hosseini, P.R. (2003). How localized consumption stabilizes predator-prey systems with finite frequency of mixing. American Naturalist 161:567-585. doi:10.1086/368293
2. Hosseini, P.R., Dobson, A. & Dhondt, A.A. (2004). Seasonality and wildlife disease: How seasonal birth, aggregation and variation in immunity affect the dynamics of Mycoplasma gallisepticum in House Finches. Proceedings of the Royal Society of London: Biological Sciences. 271:2569-2577. doi:10.1098/rspb.2004.2938
3. Hosseini, P.R. (2006) Pattern Formation and Individual-Based Models: The Importance of Understanding Individual-Based Movement. Ecological Modeling 194: 357-371. doi:10.1016/j.ecolmodel.2005.10.041
4. Seabloom, E.W., Hosseini, P.R., Power, A.G., Borer, E.T. (2009). Causes and implications of co-infection by RNA viruses in natural grasslands. American Naturalist. 173:E79-E98. doi: 10.1086/596529
5. Hosseini, P.R., Sokolow, S.H., Vandegrift, K.J., Kilpatrick, A.M. & Daszak, P. (2010). Predictive power of air travel and socio-economic data for early pandemic spread PLoS One. 5(9):e12763. doi:10.1371/journal.pone.0012763.

Additional recent publications of importance to the field

1. Campbell, S.P., Clark, A., Crampton, L., Guerry, A.D., Hatch, L.T., Hosseini, P.R., Lawler, J.J., O'Connor R.J. (2002). An assessment of monitoring efforts in endangered species recovery plans. Ecological Applications. 12:674-681. doi:10.1890/1051-0761(2002)012[0674:AAOMEI]2.0.CO;2
2. Kollias, G.V., Sydenstricker, K.V., Kollias, H.W., Ley, D.H., Hosseini, P.R., Connolly, V. & Dhondt, A.A. (2004). Experimental infection of individually caged House Finches with Mycoplasma gallisepticum. J. Wildlife Diseases. 40: 79-86.
3. Dhondt, A.A., Altizer, S., Cooch, E.G., Davis, A.K., Dobson, A., Driscoll, M.J.L., Hartup, B.K., Hawley, D. M., Hochachka, W.M., Hosseini, P.R., Jennelle, C.S., Kollias, G.V., Ley, D.H., Swarthout, E.C.H., Sydenstricker, K.V. (2005). Dynamics of a novel pathogen in an avian host: Mycoplasmal conjunctivitis in house finches. Acta Tropica 94(1):77-93. doi:10.1016/j.actatropica.2005.01.009
4. Altizer, S., Dobson, A., Hosseini, P., Hudson, P. Pascual, M., & Rohani, P. (2006). Seasonality and the dynamics of infectious diseases. Ecology Letters 9:467-484. doi:doi:10.1111/j.1461-0248.2005.00879.x
5. Hosseini, P.R., Dhondt, A.A., & Dobson, A.P. (2006). Spatial Spread of an Emerging Infectious Disease: Conjunctivitis in House Finches – Seasonal Rates and Geographic Barriers, Ecology. 87: 3037–3046. esajournals.org.
6. Borer, E., Hosseini, P.R., Seabloom, E., & Dobson, A.P. (2007). Pathogen-induced reversal of native dominance in a grassland community PNAS. 104:5473-5478 doi:10.1073/pnas.0608573104
7. Ballantyne, F.,Menge, D., Ostling, A., & Hosseini, P.R. (2008). Nutrient recycling affects autotroph and ecosystem stoichiometry, American Naturalist. 171:511-523. doi:10.1086/528967
8. Barseghian, D., Altintas, I., Jones, M. B., Crawl, D., Potter, N., Gallagher, J., Cornillon, P., Schildhauer, M., Borer, E.T., Seabloom, E.W. & Hosseini, P.R. (2009). Workflows and extensions to the Kepler scientific

workflow system to support environmental sensor data access and analysis. Ecological Informatics. 5(1):42-50 doi:10.1016/j.ecoinf.2009.08.008

9. Brandt, A.J., Seabloom, E.W., & Hosseini, P.R. (2009). Phylogeny and provenance affect plant-soil feedbacks in invaded California grasslands. Ecology 90:1063-1072.
10. Moore, S.M., Borer, E.T., Hosseini, P.R. (2010). Predators indirectly control vector-borne disease: linking predator-prey and host-pathogen models, Journal of the Royal Society Interface. 7:161-176 doi:10.1098/rsif.2009.0131

D. Research Support

Ongoing Research Support

NSF EF-1015791 Mitchell (PI) 07/01/10 – 6/30/15
National Science Foundation/National Institutes of Health: Ecology of Infectious Diseases program. The community ecology of viral pathogens – Causes and consequences of coinfection in hosts and vectors. To conduct mathematical modeling and fieldwork to understand implications in a wild grass, aphid-vectored disease system.
Role: Co-PI

NSF Daszak (PI) 06/21/10 - 06/20/15
Collaborative research: the community ecology of viral pathogens - causes and consequences of coinfection in hosts and vectors.
Role: Co-PI

GHN-A-00-09-00010-00 Morse (PI) 10/1/09-09/30/14
USAID Emerging Pandemic Threats
PREDICT - Wildlife SMART Surveillance
Modeling hotspots for disease emergence and conducting surveillance in wildlife in hotspots for new emerging zoonoses
Role: Hotspots Modeler

National Institutes Of Health Daszak (PI) 09/17/12 - 08/31/13
Fogarty International Center
Comparative Spillover Dynamics of Avian Influenza in Endemic Countries
Role: Co-PI

Completed Research Support

NIH 3R01TW005869-07S1 Daszak (PI) 07/01/10 – 06/30/11
Research: The Ecology, Emergence and Pandemic Potential of Nipah virus in Bangladesh, Supplement: Understanding and predicting the spread of H5N1 in Bangladesh, China and Globally, Modeling Research Award. To conduct model development and research to understand the role of wild and domestic poultry and livestock in creating the conditions that allow sustained spillover of human-pathogenic influenza viruses into people.
Role: Key Personnel

NIH 3R01TW005869-07S2 Daszak (PI) 07/01/10 – 06/30/11
Research: The Ecology, Emergence and Pandemic Potential of Nipah virus in Bangladesh, Supplement: Understanding and predicting the spread of H5N1 in Bangladesh, China and Globally, Field Research Award. To conduct fieldwork to understand the role of wild and domestic poultry and livestock in creating the conditions that allow sustained spillover of human-pathogenic influenza viruses into people.
Role: Key Personnel

NIH 3R01TW005869-06S4 Daszak (PI) 07/01/09 – 06/30/10
Research: The Ecology, Emergence and Pandemic Potential of Nipah virus in Bangladesh, Supplement: Understanding and predicting the spread of H5N1 in Bangladesh, China and Globally, Modeling Research

Award. To conduct model development and research to understand the role of wild and domestic poultry and livestock in creating the conditions that allow sustained spillover of human-pathogenic influenza viruses into people.
Role: Key Personnel

NIH 3R01TW005869-06S3 Daszak (PI) 07/01/09 – 06/30/10
Research: The Ecology, Emergence and Pandemic Potential of Nipah virus in Bangladesh, Supplement: Understanding and predicting the spread of H5N1 in Bangladesh, China and Globally, Field Research Award. To conduct field work to understand the role of wild and domestic poultry and livestock in creating the conditions that allow sustained spillover of human-pathogenic influenza viruses into people.
Role: Key Personnel

NSF EID 05-25666 Borer (PI) 09/01/05 – 8/31/10
Research: Predicting the effects of environmental change and host diversity on the dynamics of insect-vectored generalist pathogens.
Role: Key Personnel
The goal of this project was to assess a community-based strategy for reducing alcohol abuse among older individuals.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Ge, Xing Yi	Assistant Researcher
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Huazhong Agricultural University, Wuhan, China		07/05	Biotechnology
Huazhong Agricultural University, Wuhan, China		07/08	Preventive Veterinary Medicine
Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China	Ph.D	12/11	Biochemistry and Molecular Biology

A. Personal Statement

Throughout my career, I have received extensive molecular training, including deep sequencing, and collaborated in multiple publications in the field of viral genetic diversity in bats in China. I have investigated the genetic diversity of bat adeno-associated viruses and their virus-host interactions, and isolated 22 novel ssDNA viruses from bat fecal samples using inverse PCR, which were then identified to belong in the Circoviridae family. Additionally, using metagenomic analyses, I participated in the characterization of a totivirus from bat feces in China, which showed its capacity of infecting various insect cell lines, thus having a wide geographical distribution. Our most recent work on SARS-like coronaviruses in bats has shown that there are SARS-like CoVs in bats that use the ACE2 receptor, and therefore could be directly transmissible to humans. The discovery of MERS CoV shows that there are other coronaviruses, most likely from bats, that use different receptors to infect people. For this reason, we should understand the diversity of bat coronaviruses in China and determine whether they can infect people. In the current proposal, which aims to study coronaviruses in China, I will be responsible for the diagnosis, genomics and isolation of coronaviruses and for analyzing their receptor binding domains, in order to understand their viral spillover risk and geographic distribution. We have shown that our lab at Wuhan has the ability to identify and test these viruses for receptor usage, and I am confident that this study will allow us to find many other coronaviruses in nature with zoonotic potential.

B. Positions and Honors

Positions and Employment

2005-2008	Master's Training, College of Veterinary Medicine, Huazhong Agricultural University, China
2008-2011	Doctoral Training, Wuhan Institute of Virology, Chinese Academy of Sciences, China
2010	Doctoral Training, Unit of Molecular Genetics of RNA Viruses, Department of Virology, Institute Pasteur, Paris, France
2012-	Assistant Researcher, Wuhan Institute of Virology, Wuhan, China

Honors

2005	Excellent Thesis of Bachelor Degree of Hubei province
2005	Innovation Award of Huazhong Agricultural University
2007	First Prize of Excellent Graduate student
2012	CAS Presidential Scholarship (Excellence Prize)

C. Selected Peer-reviewed Publications

Most relevant to the current application

1. Li, Y., Ge X.Y., Hon C.C., Zhang, H., Zhou P., Zhang Y., Wang L.F., Shi Z. (2010). Prevalence and genetic diversity of adeno-associated viruses in bats, China. Journal of General Virology, 91(10), 2601-9.
2. Ge* X.Y., Rameix-Welti*, M.A., Gault* E., Chase, G., dos Santos Afonso, E., Picard D., Schwemmle, M., Naffakh, N. (2011). Influenza Virus Infection Induces the Nuclear Relocalization of the Hsp90 Co-Chaperone p23 and Inhibits the Glucocorticoid Receptor Response. *PLoS One*, 6(8), e23368. (*equal contribution)
3. Moisy, D., Jacob, Y., Laoide, B.M., Ge, X.Y., Baudin, F., Naffakh, N., Jestin, J.L. (2012). The HMGB1 protein binds to influenza virus nucleoprotein and promotes viral replication. Journal of Virology, 86(17), 9122-33.
4. Ge, X.Y., Li, Y., Yang X., Zhang H., Zhou P., Zhang Y., & Shi Z. (2012). Metagenomic Analysis of Viruses from the Bat Fecal Samples Reveals Many Novel Viruses in Insectivorous Bats in China. Journal of Virology, 86(8), 4620-30.
5. Wu L., Zhou, P., Ge X.Y., Wang, L.F., Baker M., Shi Z. (2013). Deep RNA sequencing reveals a complex transcriptional landscape of a bat adenovirus. Journal of Virology, 87(1), 503-11.

Additional recent publications of importance to the field (in chronological order)

1. Li, Y., Ge, X.Y., Zhang, H., Zhou, P., Zhu, Y., Zhang, Y., Yuan, J., Wang, L.F., Shi, Z. (2010). Host range, prevalence, and genetic diversity of adenoviruses in bats. Journal of Virology, 84(8), 3889-97.
2. Zhang, Y., Zhang, H., Dong, X., Yuan, J., Zhang, H., Yang, X., Zhou, P., Ge, X.Y., Li, Y., Wang, L.F., Shi, Z. (2010). Hantavirus outbreak associated with laboratory rats in Yunnan, China. Infection, Genetics and Evolution, 10(5), 638-44.
3. Ge, X.Y., Li, J., Peng, C., Wu, L., Yang, X., Wu, Y., Zhang, Y., Shi, Z. (2011). Genetic diversity of novel circular ssDNA viruses in bats in China. Journal of General Virology, 92, 2646–2653.
4. Yang, X., Zhang, Y., Ge, X.Y., Yuan, J., Shi, Z. (2012). A novel totivirus-like virus isolated from bat guano. Archives of Virology, 157(6), 1093-9.

D. Research Support

Ongoing Research Support

Completed Research Support

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Zhu, Gunagjian	Assistant Researcher
eRA COMMONS USER NAME (credential, e.g., agency login) xxxx	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
East China Normal University, Shanghai, China	B.S.	07/03	Biology Science
Hainan Normal University, Haikou, China	M.S.	07/03	Ecology
East China Normal University, Shanghai, China	Ph.D	6/12	Biochemistry and Molecular Biology

A. Personal Statement

Throughout my graduate studies and work with East China Normal University, I have carried out molecular biology and field ecology research focused on bat genetics and viral diversity. I have co-authored multiple publications in the field of viral genetics and bat ecology under the mentorship of Dr. Shuyi Zhang. I have also worked actively with EcoHealth Alliance on the USAID- EPT PREDICT program as a field team leader for China. For this program I have been responsible for the identification of high-risk interfaces between wildlife and people, where close contact might allow for zoonotic pathogen spillover (e.g. live animal markets). I have also led wildlife surveys which involved bat and rodent capture and sampling for viral discovery. Through this work we have conducted site-selection and wild and domestic animal sampling in Guangxi, Yunnan, Guangdong and Shanghai, and have compiled archived and current samples from birds in Shanghai Chongming Reserve for H7N9 avian influenza analyses. Under the USAID PREDICT program I collected several hundred bat samples which have been tested for coronaviruses (and several other viral families) at the Wuhan Institute of Virology. Under this current proposal, I would be responsible for developing and leading a wildlife team to sample bats, rodents, and other small mammals in the live animal markets of southern China. Through my graduate and professional work I have developed expertise in collecting high-quality, non-destructive samples from wildlife as well as expertise in molecular diagnostics. This combination of experiences allows me to understand the whole process of bringing samples from field to lab with an understanding of how to maximize opportunity for viral detection. I think that the aims of this proposal are important for providing the most current information about viral dynamics in live animal markets in China, particularly in rural areas where wildlife trade still occurs and where there is little data on spillover. I am very enthusiastic about participating in this study and confident that it has the right experts and study plan to succeed.

B. Positions and Honors

Positions and Employment

2007- Assistant Researcher, Guangdong Entomological Institute, China

Other Experiences and Professional Memberships

Honors

2009 Biology Prize of the 2009 Ig Nobel Prize

C. **Selected Peer-reviewed Publications**

Most relevant to the current application

1. Zhu, G., Han, N., Hong, T., Tan, M., Yu, D., Zhang, L. (2008). Echolocation Call, Roost and ND 1 Sequence Analysis of New Record of Nyctalus plancyi （Chiroptera： Vespertilionidae) on Hainan Island. Zoological Research, 29(4), 447-451. （in Chinese)
2. Zhu, G., Li, D., Ye, J., Hong, T., Zhang, L. (2008). New Record of Ia io in Hainan Island, its Echolocation Pulses and ND1 Analysis. Chinese Journal of Zoology, 43(5), 69-75. (in Chinese)
3. Sun, Y., Yu, D., Zhu, G., Liu, X., Zhang, S.Y., Chen, J. (2009). Isolation and characterization of 11 microsatellite loci in *Scotophilus kuhlii* (Lesser Asiatic Yellow House Bat). Conservation Genetics, 10, 1857-1859.
4. Mao, X., Zhu, G., Zhang, S.Y., Rossiter, S.J. (2010). Pleistocene climatic cycling drives intra-specific diversification in the intermediate horseshoe bat (*Rhinolophus affinis*) in Southern China. Molecular Ecology, 19(13), 2754-2769.
5. Hua, P., Zhang, L., Zhu G., Jones, G., Zhang, S., Rossiter, S.J. (2011). Hierarchical polygyny in multiparous lesser flat-headed bats. Molecular Ecology, 20(17), 3669-3680.

Additional recent publications of importance to the field (in chronological oder)

1. Zhu, G, Tang, Z., Liang, B., Zhang, X. (2007). Diet and Roost Site of Cynopterus sphinx in Winter in Haikou. Chinese Journal of Zoology, 42(4), 22-27. (in Chinese)
2. Zhang, L., Zhu, G, Jones, G., Zhang, S.Y. (2009). Conservation of bats in China: problems and recommendations. ORYX, 43(2), 179-182.
3. Tan, M., Jones, G., Zhu, G., Ye, J., Hong, T., Zhou, S., Zhang, S., Zhang, L. (2009). Fellatio by fruit bats prolongs copulation time. PLoS One, 4(10), e7595.
4. Ma, J., Jones, G., Zhu, G., Metzner, W. (2010). Echolocation behaviours of the Japanese pipistrelle bat *Pipistrellus abramus* during foraging flight. Acta Theriologica, 55(4), 315-332.
5. Zhu, G., Chmura, A., Zhang, L. (2011). Morphology, echolocation calls and diet of *Scotophilus kuhlii* (Chiroptera: Vespertilionidae) on Hainan Island, south China. Acta Chiropterologica, 14(1), 175-181.
6. [redacted] (b) (4)

D. Research Support

styles 6/5/13 2:21 PM
Comment [1]: Other support?

Ongoing Research Support

GHN-A-00-09-00010-00 Morse (PI) 10/01/09-09/30/14
PREDICT-Wildlife SMART Surveillance/PREDICT Project to pre-empt at the earlier stages possible, zoonotic diseases that impose significant threat to public health.
Role: Field Team Leader

Completed Research Support

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Zhang, Yun-Zhi	POSITION TITLE Chief Physician, Professor
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
School of Life Sciences, Yunnan University	B.S.	1990	Endemic Diseases
Kunming Medical University	M.D.	2005	Medicine
Wuhan Institute of Virology, Chinese Academy of Sciences	PhD	2010	Virology

A. Personal Statement My career in public health is focused on virology and surveillance for zoonotic infections, including hantaviruses, henipaviruses and coronaviruses. As head of infectious disease surveillance at Yunnan CDC, I am particularly interested in the risk of new pathogens emerging through the wildlife trade, which Yunnan is on the front line of in China. Through collaborative research with Wuhan Institute of Virology (Zhengli Shi) and EcoHealth Alliance (Peter Daszak, Jon Epstein and Kevin Olival), we have conducted specific surveillance in bats, rodents and primates in Yunnan Province, and on the border with Myanmar, Laos and Vietnam. This has led to our discovery of numerous CoVs in mammals, including bats, and including the recent finding of a bat SL-CoV that uses ACE2. Given my collaboration with this group, and our capacity to do extensive surveillance of wildlife and people in Yunnan, I believe that this project will generate substantial results and help us understand the risk of CoV emergence from wildlife much better in the future.

B. Positions and Honors

Positions and Employment

2003-6	Deputy chief physician, Public Health Branch of the Chinese Medical Association Youth Committee
2006-9	Head of infectious disease surveillance, Yunnan Center for Disease Control, Peoples' Republic of China.
2009-Present	Head of Infectious disease surveillance, Yunnan Institute of Endemic Disease Control and Prevention, Peoples' Republic of China

Other Experience and Professional Memberships

2002-2004	Participant, international Field Epidemiology Training Program (FETP)

C. Selected Peer-reviewed Publications

Most relevant to the current application

1. Li, Y., Wang, J.M., Hickey, A.C., Zhang, Y.Z., Li, Y., Wu, Y., Zhang, H., Yuan, J., Han, Z.G., McEachern, J., Broder, C.C., Wang, L.F. & Shi, Z. (2008). Antibodies to Nipah or Nipah-like viruses in bats, China. Emerging Infectious Diseases,14(12):1974-1976

2. Zhang, Y.Z., Zhang, H.L., Dong, X.Q., Yuan, J.F., Zhang, H.J., Yang, X.L., Zhou, P., Ge, X.Y., Li, Y., Wang, L.F., Shi, Z.L. (2010). Hantavirus outbreak associated with laboratory rats in Yunnan, China. Infection, Genetics and Evolution, 10(5):638-644
3. Li, Y., Ge, X., Zhang, H., Zhou, P., Zhu, Y., Zhang, Y.Z., Yuan, J., Wang, L.F. & Zhengli, S. (2010). Host range, prevalence, and genetic diversity of adenoviruses in bats. Journal of Virology, 84(8):3889-3897
4. Li, Y., Ge, X., Hon, C.C., Zhang, H., Zhou, P., Zhang, Y.Z., Wu, Y., Wang, L.F. & Shi, Z. (2010). Prevalence and genetic diversity of adeno-associated viruses in bats, China. Journal of General Virology, 91(10):2601-2609
5. Zhang, Y.Z., Yuan, J., Yang, X., Zhou, J., Yang, W., Peng, C., Zhang, H.L., Shi, Z. (2011). Novel Hantavirus detected in Yunnan Red-backed Vole Eothenomys miletus, China. Journal of General Virology, 92(3):1454-1457.

Additional recent publications of importance to the field (in chronological order)

1. Yuan, J.F., Zhang, Y.J., Li, J.L., Zhang, Y.Z., Wang, L.F., Shi, Z.L. (2012). Serological evidence of ebolavirus infection in bats, China. Virology Journal, 9: 236; doi: 10.1186/1743-422X-9-236
2. Yang, X.L., Zhang, Y.Z., Ge, X.Y., Yuan, J.F., Shi, Z.L. (2012). A novel totivirus-like virus isolated from bat guano. Archives of Virology, 157 (6), 1093-1099 , doi: 10.1007/s00705-012-1278-y
3. Ge, X.Y., Li, Y., Yang, XL, Zhang, H.J., Zhou, P., Zhang, Y.Z., Shi, Z.L. (2012). Metagenomic Analysis of Viruses from Bat Fecal Samples Reveals Many Novel Viruses in Insectivorous Bats in China. Journal of Virology. 86(8). 4620-4630, doi. 10.1128/JVI.06671-11

D. Research Support

Ongoing Research Support

Ministry of Science 01/01/2013- 12/01/2017
Yunnan region is an important natural reservoir of the virus and the insect vector, pathogen survey

Grant No.: 81260437 01/01/2013 -12/01/2016
National Natural Science Foundation of China
Yunnan murine viral metagenome important viral epidemic status and related research

[redacted] (b) (4) 11/01/ 2012- 11/01/2015

Grant No.: [redacted] (b) (4) 09/01/2011 -12/01/2014
Yunnan Talented young technology leaders

Completed Research Support

Grant No.: 81060132 01/01/2011-12/01/2013
National Natural Science Foundation of China,
Yunnan novel hantavirus distribution, pathogenicity and receptor research

Grant number: [redacted] (b) (4) 01/01/2011-12/01/2013
Yunnan applied basic research projects

OMB Number: 4040-0001
Expiration Date: 06/30/2011

*** ORGANIZATIONAL DUNS:** 0770900660000

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: EcoHealth Alliance, Inc.

Delete Entry *** Start Date:** 10/01/2013 *** End Date:** 09/30/2014 **Budget Period** 1

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Peter		Daszak		PD/PI		[redacted] (b) (4), (b) (6)					
2.	Dr.	Jonathan	H.	Epstein		Senior/Key Personne							
3.	Dr.	Kevin	J.	Olival		Senior/Key Personne							
4.	Dr.	Parviez	R.	Hosseini		Senior/Key Personne							
5.													
6.													
7.													
8.													

9. Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 99,498.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Scientist (tbd)	[redacted] (b) (4), (b) (6)					
1	Program Coordinator						
2	**Total Number Other Personnel**					**Total Other Personnel**	122,378.00

Total Salary, Wages and Fringe Benefits (A+B) 221,876.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS: 0770900660000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: EcoHealth Alliance, Inc.

Delete Entry | * Start Date: 10/01/2013 | * End Date: 09/30/2014 | Budget Period 1

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	Total funds requested for all equipment listed in the attached file	
	Total Equipment	

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	3,605.00
2.	Foreign Travel Costs	32,313.00
	Total Travel Cost	35,918.00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees — Total Participant/Trainee Support Costs	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 1

Next Period

* ORGANIZATIONAL DUNS: 0770900660000

* Budget Type: [X] Project [ ] Subaward/Consortium

Enter name of Organization: EcoHealth Alliance, Inc.

Delete Entry | Start Date: 10/01/2013 | * End Date: 09/30/2014 | Budget Period

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	21,400.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	227,663.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Shipping & Communications	10,000.00
9.	
10.	
Total Other Direct Costs	259,063.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	516,857.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	EcoHealth Alliance F&A Rate	44.10	289,195.00	127,535.00
2.	EcoHealth Alliance F&A on 2 Subawar	44.10	50,000.00	22,050.00
3.				
4.				
			Total Indirect Costs	149,585.00

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	666,442.00

J. Fee	Funds Requested ($)

K. * Budget Justification 1239-EHA_NIAID_COV_BUDGETJUSTIFICATION

Add Attachment | Delete Attachment | View Attachment

(Only attach one file.)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 0770900660000

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: EcoHealth Alliance, Inc.

Delete Entry *** Start Date:** 10/01/2014 *** End Date:** 09/30/2015 **Budget Period** 2

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Peter		Daszak		PD/PI							(b) (4), (b) (6)
2.	Dr.	Jonathan	H.	Epstein		Senior/Key Personne							
3.	Dr.	Kevin	J.	Olival		Senior/Key Personne							
4.	Dr.	Parviez	R.	Hosseini		Senior/Key Personne							
5.													
6.													
7.													
8.													

9. **Total Funds requested for all Senior Key Persons in the attached file**

Total Senior/Key Person 105,265.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Scientist						(b) (4), (b) (6)
1	Program Coordinator						
2	**Total Number Other Personnel**					**Total Other Personnel**	129,468.00

Total Salary, Wages and Fringe Benefits (A+B) 234,733.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

* ORGANIZATIONAL DUNS: 0770900660000

* Budget Type: [X] Project [ ] Subaward/Consortium

Enter name of Organization: EcoHealth Alliance, Inc.

Delete Entry * Start Date: 10/01/2014 * End Date: 09/30/2015 Budget Period 2

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	Total funds requested for all equipment listed in the attached file	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel	**Funds Requested ($)**
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	3,605.00
2. Foreign Travel Costs	32,313.00
Total Travel Cost	35,918.00

E. Participant/Trainee Support Costs	**Funds Requested ($)**
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 2

Next Period

* ORGANIZATIONAL DUNS: 0770900660000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: EcoHealth Alliance, Inc.

Delete Entry | Start Date: 10/01/2014 | * End Date: 09/30/2015 | Budget Period 2

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	19,250.00
2. Publication Costs	2,600.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	211,699.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Shipping and Communications	10,000.00
9. Local Reimbursement	950.00
10.	
Total Other Direct Costs	244,499.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	515,150.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	EcoHealth Alliance F&A	44.10	303,450.00	133,822.00
2.	EcoHealth Alliance F&A on 2 Subawar	44.10	50,000.00	22,050.00
3.				
4.				
			Total Indirect Costs	155,872.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	671,022.00

J. Fee	Funds Requested ($)

K. * Budget Justification 1239-EHA_NIAID_COV_BUDGETJUSTIFICATION | Add Attachment | Delete Attachment | View Attachment
(Only attach one file.)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 0770900660000

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: EcoHealth Alliance, Inc.

Delete Entry *** Start Date:** 10/01/2015 *** End Date:** 09/30/2016 **Budget Period** 3

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Peter		Daszak		PD/PI							(b) (4), (b) (6)
2.	Dr.	Jonathan	H.	Epstein		Senior/Key Personne							
3.	Dr.	Kevin	J.	Olival		Senior/Key Personne							
4.	Dr.	Parviez	R.	Hosseini		Senior/Key Personne							
5.													
6.													
7.													
8.													

9. Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 111,357.00

Additional Senior Key Persons: Add Attachment | Delete Attachment | View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Scientist						(b) (4), (b) (6)
1	Program Coordinator						
2	**Total Number Other Personnel**					**Total Other Personnel**	136,961.00

Total Salary, Wages and Fringe Benefits (A+B) 248,318.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

* ORGANIZATIONAL DUNS: 0770900660000

* Budget Type: [X] Project [ ] Subaward/Consortium

Enter name of Organization: EcoHealth Alliance, Inc.

Delete Entry | * Start Date: 10/01/2015 | * End Date: 09/30/2016 | Budget Period 3

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	Total funds requested for all equipment listed in the attached file	
	Total Equipment	

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

D. Travel	**Funds Requested ($)**
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	3,605.00
2. Foreign Travel Costs	32,313.00
Total Travel Cost	35,918.00

E. Participant/Trainee Support Costs	**Funds Requested ($)**
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 3

Next Period

* ORGANIZATIONAL DUNS: 0770900660000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: EcoHealth Alliance, Inc.

Delete Entry | Start Date: 10/01/2015 | * End Date: 09/30/2016 | Budget Period 3

F. Other Direct Costs		Funds Requested ($)
1.	Materials and Supplies	7,250.00
2.	Publication Costs	2,600.00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	213,238.00
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Shipping and Communications	7,500.00
9.	Local Reimbursement	950.00
10.		
	Total Other Direct Costs	231,538.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	515,774.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	EcoHealth Alliance F&A Rate	44.10	302,536.00	133,418.00
2.	EcoHealth Alliance F&A Rate on 2 Sub	44.10	50,000.00	22,050.00
3.				
4.				
	Total Indirect Costs			155,468.00

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	671,242.00

J. Fee	Funds Requested ($)

K. * Budget Justification 1239-EHA_NIAID_COV_BUDGETJUSTIFICATION | Add Attachment | Delete Attachment | View Attachment

(Only attach one file.)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** 0770900660000

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: EcoHealth Alliance, Inc.

Delete Entry *** Start Date:** 10/01/2016 *** End Date:** 09/30/2017 **Budget Period** 4

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Peter		Daszak		PD/PI							(b) (4), (b) (6)
2.	Dr.	Jonathan	H.	Epstein		Senior/Key Personne							
3.	Dr.	Kevin	J.	Olival		Senior/Key Personne							
4.	Dr.	Parviez	R.	Hosseini		Senior/Key Personne							
5.													
6.													
7.													
8.													

9. **Total Funds requested for all Senior Key Persons in the attached file**

Total Senior/Key Person 116,925.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Scientist						(b) (4), (b) (6)
1	Program Coordinator						
2	**Total Number Other Personnel**					**Total Other Personnel**	143,808.00

Total Salary, Wages and Fringe Benefits (A+B) 260,733.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD

* ORGANIZATIONAL DUNS: 0770900660000

* Budget Type: [X] Project [ ] Subaward/Consortium

Enter name of Organization: EcoHealth Alliance, Inc.

Delete Entry * Start Date: 10/01/2016 * End Date: 09/30/2017 Budget Period 4

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	Total funds requested for all equipment listed in the attached file	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	3,605.00
2.	Foreign Travel Costs	32,313.00
	Total Travel Cost	35,918.00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees — Total Participant/Trainee Support Costs	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 4

Next Period

* ORGANIZATIONAL DUNS: 0770900660000

* Budget Type: [X] Project [ ] Subaward/Consortium

Enter name of Organization: EcoHealth Alliance, Inc.

Delete Entry | Start Date: 10/01/2016 | * End Date: 09/30/2017 | Budget Period 4

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	7,000.00
2. Publication Costs	2,600.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	201,422.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Shipping & Communications	6,250.00
9. Local Reimbursement	950.00
10.	
Total Other Direct Costs	218,222.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	514,873.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	EcoHealth Alliance F&A	44.10	313,452.00	138,232.00
2.	EcoHealth Alliance F&A on 2 Subawar	44.10	50,000.00	22,050.00
3.				
4.				
	Total Indirect Costs			160,282.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	675,155.00

J. Fee	Funds Requested ($)

K. * Budget Justification 1239-EHA_NIAID_COV_BUDGETJUSTIFICATION | Add Attachment | Delete Attachment | View Attachment
(Only attach one file.)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

* **ORGANIZATIONAL DUNS:** 0770900660000

* **Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: EcoHealth Alliance, Inc.

Delete Entry * **Start Date:** 10/01/2017 * **End Date:** 09/30/2018 **Budget Period** 5

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Peter		Daszak		PD/PI		[redacted]					(b) (4), (b) (6)
2.		Jonathan	H.	Epstein		Senior/Key Personne							
3.		Kevin	J.	Olival		Senior/Key Personne							
4.		Parviez	R.	Hosseini		Senior/Key Personne							
5.													
6.													
7.													
8.													

9. **Total Funds requested for all Senior Key Persons in the attached file**

Total Senior/Key Person 122,772.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Scientist	[redacted]					(b) (4), (b) (6)
1	Program Coordinator						
2	**Total Number Other Personnel**					**Total Other Personnel**	150,999.00

Total Salary, Wages and Fringe Benefits (A+B) 273,771.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

* ORGANIZATIONAL DUNS: 0770900660000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: EcoHealth Alliance, Inc.

Delete Entry * Start Date: 10/01/2017 * End Date: 09/30/2018 Budget Period 5

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	Total funds requested for all equipment listed in the attached file	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	3,605.00
2. Foreign Travel Costs	32,313.00
Total Travel Cost	35,918.00

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees — Total Participant/Trainee Support Costs	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 5

* ORGANIZATIONAL DUNS: 0770900660000

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: EcoHealth Alliance, Inc.

Delete Entry | Start Date: 10/01/2017 | * End Date: 09/30/2018 | Budget Period 5

F. Other Direct Costs

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	3,500.00
2. Publication Costs	2,600.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	191,576.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Shipping & Communications	6,250.00
9. Local Reimbursement	550.00
10.	
Total Other Direct Costs	204,476.00

G. Direct Costs

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	514,165.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	EcoHealth Alliance F&A	44.10	322,588.00	142,262.00
2.	EcoHealth Alliance F&A on 2 Subawar	44.10	50,000.00	22,050.00
3.				
4.				
			Total Indirect Costs	164,312.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	678,477.00

J. Fee

J. Fee	Funds Requested ($)

K. * Budget Justification: 1239-EHA_NIAID_COV_BUDGETJUSTIFICATION | Add Attachment | Delete Attachment | View Attachment
(Only attach one file.)

Tracking Number:GRANT11418584 Funding Opportunity Number:PA-11-260 Received Date:2013-06-05T18:36:48-04:00

ECOHEALTH ALLIANCE BUDGET JUSTIFICATION

A. Key personnel:

The PD/PI, Dr. Peter Daszak, will commit (b) (4), (b) (6) per year in each year of this budget. He will be primarily responsible for overseeing the project, general management, communication and collaboration with subawardees, as well as contributing to data analysis and manuscript writing.

Senior/Key Personnel, Dr. Epstein, will commit (b) (4), (b) (6) per year. Dr. Epstein will lead the design and implementation of the bat epidemiology fieldwork; supervise field teams, lab data analyses as well as conduct field training. He will participate in regular conference calls, help with data analysis, and draft manuscripts.

Senior/Key Personnel, Dr. Olival, will commit (b) (4), (b) (6) per year. Dr. Olival will direct the bat population genetics work, assist with data analyses, and manuscript writing. He will also advise on the modeling and provide training for field teams.

Senior/Key Personnel, Dr. Hosseini, will commit (b) (4), (b) (6) per year. Dr. Hosseini will perform spatial analyses and data mapping in collaboration with of Dr. Epstein and Dr. Olival.

B. Other personnel:

A research scientist will be hired at 12 months time per year to provide direct assistance and oversight of field activities in China; maintain equipment and logistics; and coordinate animal and human sample shipment to the labs in China and in the US. This person will be based at EcoHealth Alliance, but will spend significant time in the field.

Mr. Aleksei Chmura (b) (4), (b) (6) per year) in Y1-Y5 will fulfill program assistance duties as well as conduct field research in China. Mr Chmura will coordinate regular calls, reports, maintain EcoHealth Alliance budget and financial reporting, draft subcontracts, and set-up project database advise field activities and assist with statistical analysis.

Once we secure IRBs for human sampling in Y1, we will hire three medical officers from China provincial CDCs as consultants to work in Guangxi, Hunan, and Fujian during Y2-Y5. These medical officers will be responsible for all IRB approved human sampling as well as maintaining cold chain for storage and shipping of samples.

For all EcoHealth Alliance personnel that will have salary covered by this grant, we have included the EcoHealth Alliance 5% per annum increase in salary.

C. Fringe benefits.

Fringe benefits are calculated for EcoHealth Alliance's federally approved rate of 30% of base salary.

D. Consultant: Once all permits are in place in Y2-5, EcoHealth Alliance will contract three technician-consultants trained in phlebotomy – one in each province: Guangxi, Hunan, and Fujian. The technicians will conduct interviews as part of the human wildlife contact survey as well as collect blood samples from volunteers in animal markets. These will be given daily rate of $67 for 5 months work per year (3 technicians x 5

months x 20 days per month x $67 = $20,000 per year) as well as funds to cover shipping and maintaining cold-chain ($333 x 3 months x 3 provinces = $3,000 per year) from provincial areas to Wuhan Institute of Virology. We also will support the technician's allowable room/transportation/food costs expected to average monthly at food ($62), room ($100), and transportation ($100): $262 x 3 technicians x 5 months = $3,923 per year.

E. Equipment: N/A

F. Supplies: For Y1-Y5, we request annual support for tubes, syringes (5k); computer, phone, GPS (8k); lab reagents and buffer (10k); shipping (10k); PPE (10k); bat catching equipment (10k); food/accomodation for field team (10k); dry shippers, liquid Nitrogen (8k)

G. Travel

Domestic travel: $4,400 is requested for years 1-5, comprising $2,200 each for the PI (Dr. Daszak) and Senior/Key Personnel (Dr. Epstein) for travel to collaborating labs, to group meetings, and domestic scientific conferences to present results of our work.

International travel: $33,000 is requested p.a. in Years 1-5. This will support 4 RT flights p.a. from New York to Shanghai or Guangzhou for the field veterinarian; 3 for the Senior/Key Personnel and 1 for the PI (Daszak) @ $2500 ea; Food and accommodation at $8,500 p.a. for Senior/Key Personnel and the field team in China including field activities. Field vehicle rental & driver hire $500/wk x 9 wks.

H. Participant support costs: We are requesting consortium/contractual support for our two partners: East China Normal University and Wuhan Institute of Virology. We are requesting $34,560 per year for Y1-Y5 for East China Normal University and for Wuhan Institute of Virology $93,960 in Y1 and $81,000 in Y2-Y5. These amounts are justified as follows:

EAST CHINA NORMAL UNIVERSITY

EcoHealth Alliance Budget Justification, H. Participant Support Costs (ctd)

a) Senior Personnel: Dr. Shuyi Zhang (b) (4), (b) (6) per year in Y1-Y5 Dr. Zhang will oversee the field sample collection and coordination of sample transfer to Wuhan or US partners. He will not request any salary from this grant. His salary will be covered by his instiutional discretionary funds.

b) Other personnel: A full time field biologist, Dr. GuanJian Zhu (b) (4), (b) (6) per year), will implement field site visits, sample collection, and sample shipment to Wuhan or to Co-PIs. A full time field technician, Mr. Junpeng Zhang (b) (4), (b) (6) per year), will assist with sample collection, handling, and transport from field to lab as well as with sample shipping from East China Normal University to Wuhan or US partners. Both Mr. Zhang and Dr. Zhu will work full-time for in Y1-Y5.

c) Fringe Benefits: Fringe benefits are provided at ECNU rate of 5% to Dr. Zhu and Mr. Zhang in Y1-Y5.

d) Equipment: N/A

e) Supplies: $560 are allocated per year in Y1-Y5 to support Field Biologist and Field Technician costs for telephone internet, and GPS: Phone = 15x2= 30; Internet = 5x2 = 10, GPS (batteries) = 3.5x2 = 10; total per month = $47.

f) Travel: Dr. Zhang will provide travel costs for Field Biologist and Field Technician from discretionary funding. We request $2,000 per year to support Dr. Zhang and Dr. Zhu to travel to US for Co-Investigator meetings in either Boston or New York. Support will provide room and board for the two at per diem rate of $250 for 4 days (= 250x4x2 = $2,000). Dr. Zhang is already supported for travel funds of his own in those years.

g) Participant support costs: N/A

h) Other direct costs: N/A

i) Indirect Costs. All administrative costs are charged directly.

WUHAN INSTITUTE OF VIROLOGY

a) Key personnel. Dr. Zhengli Shi, Senior Virologist. (b) (4), (b) (6) per year in Y1-Y5. Dr. Shi will oversee the coronavirus screening for all samples collected in China. She will work with the PI, Co-Investigators, and Senior/Key Personnel to analyze data and write manuscripts. She will also coordinate data and material sharing with the co-Investigators. Dr. Shi will not take salary on this grant and is funded by discretionary sources at her Institute.

b) Other personnel:
Mr. Jialu Li, Lab technician. (b) (4), (b) (6) p.a. in Y1-Y5. The lab technician will test all bat and other animal samples collected in China for coronaviruses and will conduct molecular characterization and phylogenetic analyses of new coronavirus strains identified as well as catalog and ship samples and maintain a sample database.

EcoHealth Alliance Budget Justification, H. Participant Support Costs (ctd)

c) Fringe benefits: Wuhan Institute of Virology benefit rate of 5% is applied to salary for Mr. Jialu Li in Y1-Y5.

d) Equipment: The subcontractor will purchase one ultracold -80°C freezer for dedicated sample storage for this project. $12,960.

e) Supplies: Annual costs for Laboratory reagents (20k); shipping to US (twice per year x 2.5k = 5k); testing costs (30k); expendable equipment costs (5k) are requested in Y1-Y5.

f) Travel: Round-trip airfare to Boston/NYC for Co-Investigator (Dr. Shi) for attending PD/PI meetings once-per year in Y1-Y5: $2,600. Dr. Shi has discretionary funds to supplement travel expenditures. We also request $2,400 to cover in-country transportation costs of supplies and samples.

g) Participant support costs: N/A

h) Other direct costs: We request annual support costs for Telephone ($180), Printing ($144), Conference Calls ($504), and local shipping charges ($172) for Dr. Shi's laboratory and Mr. Li in Y1-Y5.

i) Indirect Costs. All administrative costs are charged directly.

H. Other direct costs: We request $71,000 in year one for sample collection materials including bat catching equipment, syringes, tubes, and reagents, and 2 liquid nitrogen dry shippers at $3,000each. We also request $10,000 for shipping supplies from NY to China in yr 1, then $5,000 p.a. for shipping in years 2-5. We also request $4,000 in years 3 and 4 for lab reagents to complete population genetics tests.

Other expenses for publishing and communications including video conferencing facilities will be covered by EcoHealth Alliance.

I. Indirect Costs

We are requesting the EcoHealth Alliance federally-approved indirect cost rate of 30.0% on all applicable direct costs. Indirect is taken only on the first $25,000 for each consortium/contractual agreement. As there are 2 (one to Wuhan Insititute of Virology and the other to East China Normal University), a total of $15,000 ($7,500x2) is taken as indirect on consortium/contractual agreements only in Y1 and included as part of direct cost calculations. In Y2-Y5 no indirect is taken on consortium/contractual agreements.

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		555,817.00
Section B, Other Personnel		683,614.00
Total Number Other Personnel	10	
Total Salary, Wages and Fringe Benefits (A+B)		1,239,431.00
Section C, Equipment		
Section D, Travel		179,590.00
1. Domestic	18,025.00	
2. Foreign	161,565.00	
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		1,157,798.00
1. Materials and Supplies	58,400.00	
2. Publication Costs	10,400.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs	1,045,598.00	
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	40,000.00	
9. Other 2	3,400.00	
10. Other 3		
Section G, Direct Costs (A thru F)		2,576,819.00
Section H, Indirect Costs		785,519.00
Section I, Total Direct and Indirect Costs (G + H)		3,362,338.00
Section J, Fee		

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** 5290274740000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

*** Start Date:** 10-01-2013 *** End Date:** 09-30-2014 **Budget Period: 1**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Zhengli		Shi		Co-Investigator							(b) (4), (b) (6)
2.	Dr.	Xingyi		Ge		Senior Research Technician							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Laboratory Technician100						(b) (4), (b) (6)
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	

RESEARCH & RELATED Budget {A-B} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** 5290274740000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

*** Start Date:** 10-01-2013 *** End Date:** 09-30-2014 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	*** Funds Requested ($)**
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	**Funds Requested ($)**
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,060.00
2. Foreign Travel Costs	
Total Travel Cost	**2,060.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)**
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** 5290274740000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

*** Start Date:** 10-01-2013 *** End Date:** 09-30-2014 **Budget Period:** 1

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	95,737.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**95,737.00**

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	**123,699.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. Wuhan Institute of Virology F&A	8.00	123,699.00	9,896.00
		Total Indirect Costs	**9,896.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	**133,595.00**

J. Fee	**Funds Requested ($)**

K. * Budget Justification	File Name: 1242-WIV NIAID COV BUDGET JUSTIFICATION.pdf (Only attach one file.)	Mime Type: application/pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 5290274740000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

*** Start Date:** 10-01-2014 *** End Date:** 09-30-2015 **Budget Period:** 2

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Zhengli		Shi		Co-Investigator							(b) (4), (b) (6)
2.	Dr.	Xingyi		Ge		Senior Research Technician							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Laboratory Technician						(b) (4), (b) (6)
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 5290274740000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

*** Start Date:** 10-01-2014 *** End Date:** 09-30-2015 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,060.00
2. Foreign Travel Costs	
Total Travel Cost	**2,060.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 5290274740000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

*** Start Date:** 10-01-2014 *** End Date:** 09-30-2015 **Budget Period:** 2

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	100,756.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**100,756.00**

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	**128,718.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. Wuhan Institute of Virology F&A Rate	8.00	128,718.00	10,297.00
		Total Indirect Costs	**10,297.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	**139,015.00**

J. Fee	**Funds Requested ($)**

K. * Budget Justification	File Name: 1242-WIV NIAID COV BUDGET JUSTIFICATION.pdf (Only attach one file.)	Mime Type: application/pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 5290274740000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

*** Start Date:** 10-01-2015 *** End Date:** 09-30-2016 **Budget Period:** 3

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Zhengli		Shi		Co-Investigator		[redacted]					(b) (4), (b) (6)
2.	Dr.	Xingyi		Ge		Senior Research Technician							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** [redacted] (b) (4), (b)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Laboratory Technician	[redacted]					(b) (4), (b) (6)
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted] (b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	

RESEARCH & RELATED Budget {A-B} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 5290274740000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

*** Start Date:** 10-01-2015 *** End Date:** 09-30-2016 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,060.00
2. Foreign Travel Costs	
Total Travel Cost	**2,060.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 5290274740000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

*** Start Date:** 10-01-2015 *** End Date:** 09-30-2016 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	119,373.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**119,373.00**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**147,335.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Wuhan institute of Virology F&A Rate	8.00	147,335.00	11,787.00
		Total Indirect Costs	**11,787.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**159,122.00**

J. Fee	Funds Requested ($)

K. * Budget Justification	File Name: 1242-WIV NIAID COV BUDGET JUSTIFICATION.pdf (Only attach one file.)	Mime Type: application/pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** 5290274740000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

*** Start Date:** 10-01-2016 *** End Date:** 09-30-2017 **Budget Period: 4**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Zhengli		Shi		Co-Investigator							(b) (4), (b) (6)
2.	Dr.	Xingyi		Ge		Senior Research Technician							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Laboratory Technician						(b) (4), (b) (6)
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** 5290274740000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

*** Start Date:** 10-01-2016 *** End Date:** 09-30-2017 **Budget Period: 4**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,060.00
2. Foreign Travel Costs	
Total Travel Cost	**2,060.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** 5290274740000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

*** Start Date:** 10-01-2016 *** End Date:** 09-30-2017 **Budget Period: 4**

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	119,373.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**119,373.00**

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	**147,335.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. Wuhan Institute of Virology F&A Rate	8.00	147,335.00	11,787.00
		Total Indirect Costs	**11,787.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	**159,122.00**

J. Fee	**Funds Requested ($)**

K. * Budget Justification	File Name: 1242-WIV NIAID COV BUDGET JUSTIFICATION.pdf (Only attach one file.)	Mime Type: application/pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** 5290274740000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

*** Start Date:** 10-01-2017 *** End Date:** 09-30-2018 **Budget Period:** 5

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Zhengli		Shi		Co-Investigator							(b) (4), (b) (6)
2.	Dr.	Xingyi		Ge		Senior Research Technician							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Laboratory Technician						(b) (4), (b) (6)
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	

RESEARCH & RELATED Budget {A-B} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** 5290274740000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

*** Start Date:** 10-01-2017 *** End Date:** 09-30-2018 **Budget Period: 5**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,060.00
2. Foreign Travel Costs	
Total Travel Cost	**2,060.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** 5290274740000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

*** Start Date:** 10-01-2017 *** End Date:** 09-30-2018 **Budget Period:** 5

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	119,373.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**119,373.00**

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	**147,335.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. Wuhan Institute of Virology F&A Rate	8.00	147,355.00	11,787.00
		Total Indirect Costs	**11,787.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	**159,122.00**

J. Fee	**Funds Requested ($)**

K. * Budget Justification	File Name: 1242-WIV NIAID COV BUDGET JUSTIFICATION.pdf (Only attach one file.)	Mime Type: application/pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		**79,325.00**
Section B, Other Personnel		**50,185.00**
Total Number Other Personnel	5	
Total Salary, Wages and Fringe Benefits (A+B)		**129,510.00**
Section C, Equipment		
Section D, Travel		**10,300.00**
1. Domestic	10,300.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		**554,612.00**
1. Materials and Supplies	554,612.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		**694,422.00**
Section H, Indirect Costs		**55,554.00**
Section I, Total Direct and Indirect Costs (G + H)		**749,976.00**
Section J, Fee		

WUHAN INSTITUTE OF VIROLOGY BUDGET JUSTIFICATION, SUBAWARD

A. Senior/Key Personnel:

Co-Investigator, Dr. Zhengli Shi, a Senior Research Scientist at the Chinese Academy of Science's Wuhan Institute of Virology will commit (b) (4), (b) (6) per year (b) (4), (b) (6) to this project to refine study protocols, coordinate research, oversee implementation of all activities, analyze data, lead regular meetings with other PD/PI and Other Senior/Key Personnel as well as draft papers. Dr. Xingyi Ge, Senior Research Technician, will commit (b) (4), (b) (6) per year to perform all laboratory work and directly supervise the laboratory technician.

B. Other Personnel

One laboratory technician will commit (b) (4), (b) (6) per year (b) (4), (b) (6) each to this project to perform all required laboratory assays and maintenance as well as participate in selected meetings, perform research for papers, and assist Dr. Shi in performing the work under this award.

All Wuhan Institute of Virology salaries include the US "overhead" or "fringe", so this is not calculated separately.

C. Equipment

No equipment over $5,000 will be purchased.

D. Travel

We are requesting $2,060 per year for all years for Senior/Key Personnel Dr. Shi and Dr. Ge to travel to Shanghai to visit partner laboratory at East China Normal Univeristy (ECNU) and meet with the PD/PI as well as with collaborators on this proposal: these include EcoHealth Alliance, East China Normal University, Yunnan CDC, Shanghai CDC, and Guangdong CDC. Travel is calculated at one round trip airfare from Wuhan to Shanghai ($300), three-night hotel in Shanghai ($150 per night), and four days per diem (at $70 per day)

F. Other Direct Costs

We are requesting support for laboratory experiments and related costs.

RNA Extractions

We will be running RNA Extractions for 1,000 bats per year (three samples per bat: oral, anal, and blood) in each year of the project. This will cost $13,922 per year (QIAamp ViralRNA Mini Kit with Axygen Pipette Tips and Filter Tubes at $4.64 per sample). Extracted RNA per animal will be pooled.

RT-PCR

Costs for 1-Step RT-PCR assays for Coronavirus conducted on 1,000 samples per year for each year of the project total $7,123 and are detailed as follows: Superscript III one step kit ($5.18 per sample); Platinum Tag DNA Polymerase ($0.57 per sample); nuclease-free water ($0.16 per sample); and Axygen Pipette Tips and Filter Tubes ($1.21 per sample).

DNA Sequencing

In each year of the project, DNA Sequencing will be performed on 3,200 samples at a cost of $2.91 per reaction. We request a total of $9,325 per year in each year.

Laboratory Supplies
We request support for *in vitro* infection experiments using pseudoviruses carrying the spike proteins (wild type or mutants) or live viruses in cell lines of different origins, binding affinity assays between the spike proteins (wild type or mutants) and different cellular receptor molecules, and humanized mouse experiments.

In Year 1, $65,367 is requested: Lipofectamine2000 transfection reagent at a cost of $2,428; cell lines from bats and other mammals including primates and humans at a cost of $971; *in vitro* infection experiments require GIBCO Fetal Bovine Serum ($3,562), GIBCO antibiotic antimycotic ($563), GIBCO medium ($2,914) as well as $19,426 for Corning Cell culture; receptor-mutant pseudovirus binding assays require Luciferase assay system E1500 ($858), pseudovirus package ($3,885), and sequencing ($22,664); $8,094 is required for protein expression from the binding affinity assays.

In Year 2, $70,385 is requested: Lipofectamine2000 transfection reagent at a cost of $2,428; cell lines from bats and other mammals including primates and humans at a cost of $971 – sufficient cell lines will be established by the end of Year 2, so this cost requirement will discontinue in Years 3-5; increased number of *in vitro* infection experiments require slightly more funding for GIBCO Fetal Bovine Serum ($4,047) as well as GIBCO antibiotic antimycotic ($563), GIBCO medium ($2,914) as well as $19,426 for Corning Cell culture; receptor-mutant pseudovirus binding assays require Luciferase assay system E1500 ($858), pseudovirus package requirements will approximately double from Y1 ($6,799), and sequencing ($22,664); $9,713 is required for protein expression from the increased Year 2 number of binding affinity assays at a slightly higher cost than year one as well.

In Years 3, 4 and 5, $89,002 is requested per year: Lipofectamine2000 transfection reagent at a cost of $2,428 per year; increased number of *in vitro* infection experiments require slightly more funding for GIBCO Fetal Bovine Serum ($5,828 per year) as well as GIBCO antibiotic antimycotic ($563 per year), GIBCO medium ($2,914 per year) as well as $19,426 per year for Corning Cell culture; receptor-mutant pseudovirus binding assays require Luciferase assay system E1500 ($858 per year), pseudovirus package requirements will be $6,799 per year, sequencing ($22,664 per year) and gene synthesis ($12,915 per year) will also be required; $9,713 per year is required for protein expression from binding affinity assays; in only years 3, 4, and 5 humanized mouse *in vivo* experimental animals will be raised at an annual cost of $4,857 per year.

H. Indirect Costs

We are requesting an extremely indirect cost of 8% on all direct costs.

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** 4209454950000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: East China Normal University

*** Start Date:** 10-01-2013 *** End Date:** 09-30-2014 **Budget Period:** 1

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Shuyi		Zhang		Co-Investigator							(b) (4), (b) (6)
2.	Dr.	Guangjian		Zhu		Research Techni-cian							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	**25,000.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** 4209454950000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: East China Normal University

*** Start Date:** 10-01-2013 *** End Date:** 09-30-2014 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,700.00
2. Foreign Travel Costs	
Total Travel Cost	**2,700.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** 4209454950000

*** Budget Type:** ○ Project ● Subaward/Consortium

Enter name of Organization: East China Normal University

*** Start Date:** 10-01-2013 *** End Date:** 09-30-2014 **Budget Period: 1**

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Fieldwork Support Costs	59,400.00
Total Other Direct Costs	**59,400.00**

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	**87,100.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. East China Normal University F&A Rate	8.00	87,100.00	6,968.00
		Total Indirect Costs	**6,968.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	**94,068.00**

J. Fee	**Funds Requested ($)**

K. * Budget Justification	File Name: 1243-ECNU NIAID COV BUDGET JUSTIFICATION.pdf (Only attach one file.)	Mime Type: application/pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 4209454950000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: East China Normal University

*** Start Date:** 10-01-2014 *** End Date:** 09-30-2015 **Budget Period:** 2

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Shuyi		Zhang		Co-Investigator							(b) (4), (b) (6)
2.	Dr.	Guangjian		Zhu		Research Technician							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	(b) (4), (b) (6)

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 4209454950000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: East China Normal University

*** Start Date:** 10-01-2014 *** End Date:** 09-30-2015 **Budget Period:** 2

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,700.00
2. Foreign Travel Costs	
Total Travel Cost	**2,700.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 4209454950000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: East China Normal University

*** Start Date:** 10-01-2014 *** End Date:** 09-30-2015 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Field Work Support Costs	39,600.00
Total Other Direct Costs	**39,600.00**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**67,300.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. East China Normal University F&A Rate	8.00	67,300.00	5,384.00
		Total Indirect Costs	**5,384.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**72,684.00**

J. Fee	Funds Requested ($)

K. * Budget Justification	File Name: 1243-ECNU NIAID COV BUDGET JUSTIFICATION.pdf	Mime Type: application/pdf
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 4209454950000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: East China Normal University

*** Start Date:** 10-01-2015 *** End Date:** 09-30-2016 **Budget Period:** 3

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Shuyi		Zhang		Co-Investigator							(b) (4), (b) (6)
2.	Dr.	Guangjian		Zhu		Research Technician							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	(b) (4), (b) (6)

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 4209454950000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: East China Normal University

*** Start Date:** 10-01-2015 *** End Date:** 09-30-2016 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,700.00
2. Foreign Travel Costs	
Total Travel Cost	**2,700.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 4209454950000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: East China Normal University

*** Start Date:** 10-01-2015 *** End Date:** 09-30-2016 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Field Work Support Costs	29,700.00
Total Other Direct Costs	**29,700.00**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**50,108.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. East China Normal University F&A Rate	8.00	50,108.00	4,009.00
		Total Indirect Costs	**4,009.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**54,117.00**

J. Fee	Funds Requested ($)

K. * Budget Justification File Name: 1243-ECNU NIAID COV BUDGET JUSTIFICATION.pdf Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** 4209454950000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: East China Normal University

*** Start Date:** 10-01-2016 *** End Date:** 09-30-2017 **Budget Period:** 4

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Shuyi		Zhang		Co-Investigator							(b) (4), (b) (6)
2.	Dr.	Guangjian		Zhu		Research Techni-cian							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	(b) (4), (b) (6)

RESEARCH & RELATED Budget {A-B} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** 4209454950000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: East China Normal University

*** Start Date:** 10-01-2016 *** End Date:** 09-30-2017 **Budget Period: 4**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	*** Funds Requested ($)**
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	**Funds Requested ($)**
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,700.00
2. Foreign Travel Costs	
Total Travel Cost	**2,700.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)**
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** 4209454950000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: East China Normal University

*** Start Date:** 10-01-2016 *** End Date:** 09-30-2017 **Budget Period:** 4

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Field Work Support Costs	19,800.00
Total Other Direct Costs	**19,800.00**

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	**39,167.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. East China Normal University F&A Rate	8.00	39,167.00	3,133.00
		Total Indirect Costs	**3,133.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	**42,300.00**

J. Fee	**Funds Requested ($)**

K. * Budget Justification	File Name: 1243-ECNU NIAID COV BUDGET JUSTIFICATION.pdf (Only attach one file.)	Mime Type: application/pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** 4209454950000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: East China Normal University

*** Start Date:** 10-01-2017 *** End Date:** 09-30-2018 **Budget Period:** 5

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Shuyi		Zhang		Co-Investigator							(b) (4), (b) (6)
2.	Dr.	Guangjian		Zhu		Reasearch Techni-cian							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	(b) (4), (b) (6)

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** 4209454950000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: East China Normal University

*** Start Date:** 10-01-2017 *** End Date:** 09-30-2018 **Budget Period: 5**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,700.00
2. Foreign Travel Costs	
Total Travel Cost	**2,700.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** 4209454950000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: East China Normal University

*** Start Date:** 10-01-2017 *** End Date:** 09-30-2018 **Budget Period:** 5

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Field Work Support Costs	14,850.00
Total Other Direct Costs	**14,850.00**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**30,050.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. East China Normal University F&A Rate	8.00	30,050.00	2,404.00
		Total Indirect Costs	**2,404.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**32,454.00**

J. Fee	Funds Requested ($)

K. * Budget Justification	File Name: 1243-ECNU NIAID COV BUDGET JUSTIFICATION.pdf (Only attach one file.)	Mime Type: application/pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		**96,875.00**
Section B, Other Personnel		
Total Number Other Personnel		
Total Salary, Wages and Fringe Benefits (A+B)		**96,875.00**
Section C, Equipment		
Section D, Travel		**13,500.00**
1. Domestic	13,500.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		**163,350.00**
1. Materials and Supplies		
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	163,350.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		**273,725.00**
Section H, Indirect Costs		**21,898.00**
Section I, Total Direct and Indirect Costs (G + H)		**295,623.00**
Section J, Fee		

EAST CHINA NORMAL UNIVERSITY BUDGET JUSTIFICATION, SUBAWARD

A. Senior/Key Personnel:

Co-Investigator Dr. Shu-Yi Zhang will commit (b) (4), (b) (6) per year to this project to refine field work design, coordinate research, oversee implementation of all activities, analyze data, lead regular meetings with other PD/PI and Other Senior/Key Personnel as well as draft papers. To keep costs low, Dr. Zhang will not take any salary. Research Technician, Dr. Zhu Guangjian, will commit (b) (4), (b) (6) in Years 1 and 2; 34 weeks or (b) (4), (b) (6) in Year 3; (b) (4), (b) (6) in Year 4 and only (b) (4), (b) (6) in Year 5 to directly coordinate field work, liaise with local CDCs, ensure shipment of samples to Wuhan Institute of Virology, and participate in some meetings as well as assist with drafting papers.

B. Other Personnel

There are no additional personnel.

All East China Normal University salaries include the US "overhead" or "fringe", so this is not calculated separately.

C. Equipment

No equipment over $5,000 will be purchased.

D. Travel

We are requesting $2,700 per year for all five years of this award to cover 3-per-year round-trip flights each from Shanghai, China, to Yunnan, Guangdong, and Guangxi for Dr. Zhu Guangjian to meet with collaborating institutions, train field teams, and ensure sample collection, storage, and shipments. Each flight is estimated at $300.

F. Other Direct Costs

Fieldwork Support Costs

In year 1, we are requesting $59,400 to support 12-months of fieldwork costs. This we estimate as follows: $7,200 for driver ($600 per month) and car rental ($600 per month); $2,700 for gas ($450 per month); $10,800 to support a field team of three ($600 per month); and $9,000 for meals and lodging at a rate of $50 per day ($1,500 per month).

In year 2, we are requesting $39,600 to support 8-months of fieldwork costs. This we estimate as follows: $7,200 for driver ($600 per month) and car rental ($600 per month); $2,700 for gas ($450 per month); $10,800 to support a field team of three ($600 per month); and $9,000 for meals and lodging at a rate of $50 per day ($1,500 per month).

In year 3, we are requesting $29,700 to support 24-weeks or 6-months of fieldwork costs. This we estimate as follows: $7,200 for driver ($600 per month) and car rental ($600 per month); $2,700 for gas ($450 per month); $10,800 to support a field team of three ($600 per month); and $9,000 for meals and lodging at a rate of $50 per day ($1,500 per month).

In year 4, we are requesting $19,800 to support 16-weeks or 4-months of fieldwork costs. This we estimate as follows: $7,200 for driver ($600 per month) and car rental ($600 per month); $2,700 for gas ($450 per month); $10,800 to support a field team of three ($600 per month); and $9,000 for meals and lodging at a rate of $50 per day ($1,500 per month).

In year 5, we are requesting $14,850 to support 12-weeks or 3-months of fieldwork costs. This we estimate as follows: $7,200 for driver ($600 per month) and car rental ($600 per month); $2,700 for gas ($450 per month); $10,800 to support a field team of three ($600 per month); and $9,000 for meals and lodging at a rate of $50 per day ($1,500 per month).

H. Indirect Costs

We are requesting an extremely indirect cost of 8% on all direct costs.

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.   * First Name: Peter
Middle Name:
* Last Name: Daszak
Suffix:

2. Human Subjects

Clinical Trial? ☒ No ☐ Yes

* Agency-Defined Phase III Clinical Trial? ☐ No ☐ Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix: Dr.   * First Name: Peter
Middle Name:
* Last Name: Daszak
Suffix:

* Phone Number: [redacted] (b) (6)   Fax Number: +1.212.380.4465
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* Title: President

* Street1: 460 West 34th Street
Street2: 17th Floor
* City: New York
County/Parish:
* State: NY: New York
Province:
* Country: USA: UNITED STATES   * Zip / Postal Code: 10001-2317

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/research/registry/. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

OMB Number: 0925-0001

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated for your reference, as you attach the appropriate sections of the Research Plan.

*Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)		Add Attachment	Delete Attachment	View Attachment
2. Specific Aims	1240-NIH_COv_Specific_Aims.p	Add Attachment	Delete Attachment	View Attachment
3. *Research Strategy	1241-Understanding_the_risk_	Add Attachment	Delete Attachment	View Attachment
4. Inclusion Enrollment Report		Add Attachment	Delete Attachment	View Attachment
5. Progress Report Publication List		Add Attachment	Delete Attachment	View Attachment

Human Subjects Sections

6. Protection of Human Subjects	1254-NIH_COv_Protection_Hum	Add Attachment	Delete Attachment	View Attachment
7. Inclusion of Women and Minorities	1255-NIH_COv_Inclusion_of_W	Add Attachment	Delete Attachment	View Attachment
8. Targeted/Planned Enrollment Table	1256-China_CoV_Planned_enro	Add Attachment	Delete Attachment	View Attachment
9. Inclusion of Children	1257-NIH_COv_Inclusion_of_Ch	Add Attachment	Delete Attachment	View Attachment

Other Research Plan Sections

10. Vertebrate Animals	1258-NIH_COv_Vertebrate_Anim	Add Attachment	Delete Attachment	View Attachment
11. Select Agent Research	1259-NIH_COv_Select_Agent_R	Add Attachment	Delete Attachment	View Attachment
12. Multiple PD/PI Leadership Plan		Add Attachment	Delete Attachment	View Attachment
13. Consortium/Contractual Arrangements	1260-NIH_COv_Consortium_Cont	Add Attachment	Delete Attachment	View Attachment
14. Letters of Support	1261-NIAID_COV_2013_AllSuppo	Add Attachment	Delete Attachment	View Attachment
15. Resource Sharing Plan(s)	1262-NIH_COv_Resource_Sharin	Add Attachment	Delete Attachment	View Attachment

16. Appendix Add Attachments Remove Attachments View Attachments

SPECIFIC AIMS:

Zoonotic coronaviruses are a significant threat to global health, as demonstrated with the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, and the recent emergence Middle East Respiratory Syndrome (MERS-CoV). The wildlife reservoirs of SARS-CoV were identified by our group as bat species, and since then hundreds of novel bat-CoVs have been discovered (including >260 by our group). These, and other wildlife species, are hunted, traded, butchered and consumed across Asia, creating a largescale human-wildlife interface, and high risk of future emergence of novel CoVs.

To understand the risk of zoonotic CoV emergence, we propose to examine **1)** the transmission dynamics of bat-CoVs across the human-wildlife interface, and **2)** how this process is affected by CoV evolutionary potential, and how it might force CoV evolution. We will assess the nature and frequency of contact among animals and people in two critical human-animal interfaces: live animal markets in China and people who are highly exposed to bats in rural China. In the markets we hypothesize that viral emergence may be accelerated by heightened mixing of host species leading to viral evolution, and high potential for contact with humans. In this study, we propose three specific aims and will screen free ranging and captive bats in China for known and novel coronaviruses; screen people who have high occupational exposure to bats and other wildlife; and examine the genetics and receptor binding properties of novel bat-CoVs we have already identified and those we will discover. We will then use ecological and evolutionary analyses and predictive mathematical models to examine the risk of future bat-CoV spillover to humans. This work will follow 3 specific aims:

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces. We will examine if: **1)** wildlife markets in China provide enhanced capacity for bat-CoVs to infect other hosts, either via evolutionary adaptation or recombination; **2)** the import of animals from throughout Southeast Asia introduces a higher genetic diversity of mammalian CoVs in market systems compared to within intact ecosystems of China and Southeast Asia; **We will interview people about the nature and frequency of contact with bats** and other wildlife**; collect blood samples from people highly exposed to wildlife;** and **collect a full range of clinical samples from bats and other mammals in the wild and in wetmarkets;** and screen these for CoVs using serological and molecular assays.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk. We propose two competing hypotheses: **1)** CoV host-range in bats and other mammals is limited by the phylogenetic relatedness of bats and evolutionary conservation of CoV receptors; **2)** CoV host-range is limited by geographic and ecological opportunity for contact between species so that the wildlife trade disrupts the 'natural' co-phylogeny, facilitates spillover and promotes viral evolution. We will develop CoV phylogenies from sequence data collected previously by our group, and in the proposed study, as well as from Genbank. We will examine co-evolutionary congruence of bat-CoVs and their hosts using both functional (receptor) and neutral genes. We will predict host-range in unsampled species using a generalizable model of host and viral ecological and phylogenetic traits to explain patterns of viral sharing between species. We will test for positive selection in market vs. wild-sampled viruses, and use data to parameterize mathematical models that predict CoV evolutionary and transmission dynamics. We will then examine scenarios of how CoVs with different transmissibility would likely emerge in wildlife markets.

Specific Aim 3: Testing predictions of CoV inter-species transmission. We will test our models of host range (i.e. emergence potential) experimentally using reverse genetics, pseudovirus and receptor binding assays, and virus infection experiments in cell culture and humanized mice. With bat-CoVs that we've isolated or sequenced, and using live virus or pseudovirus infection in cells of different origin or expressing different receptor molecules, we will assess potential for each isolated virus and those with receptor binding site sequence, to spill over. We will do this by sequencing the spike (or other receptor binding/fusion) protein genes from all our bat-CoVs, creating mutants to identify how significantly each would need to evolve to use ACE2, CD26/DPP4 (MERS-CoV receptor) or other potential CoV receptors. We will then use receptor-mutant pseudovirus binding assays, *in vitro* studies in bat, primate, human and other species' cell lines, and with humanized mice where particularly interesting viruses are identified phylogenetically, or isolated. These tests will provide public health-relevant data, and also iteratively improve our predictive model to better target bat species and CoVs during our field studies to obtain bat-CoV strains of the greatest interest for understanding the mechanisms of cross-species transmission.

RESEARCH STRATEGY

A. SIGNIFICANCE:

General: Severe Acute Respiratory Syndrome, like many other emerging human pathogens (*1*), originated in a wildlife reservoir host, initially thought to be terrestrial mammals (*2*), and later shown by our group to be bats (*3*). Bats harbor the most closely-related viruses to SARS-CoV, and are traded widely for food in the wildlife markets of China (*4*). The diversity of bat-CoVs is very high, and some studies even suggest that the *Coronaviridae* originated within bats (*3, 5-9*). Recently a novel CoV emerged in the Middle East (MERS-CoV) (*10*) and available data (including from our group) suggest that MERS-CoV also has bat origins (*11-13*). Given that hunting and eating of bats continues across Asia, future spillover of bat-CoVs is likely. Yet *salient questions remain: How diverse are bat-CoVs? Can the conditions in these markets enhance bat-CoV evolution and spillover of bat-CoVs?* **The proposed work** addresses these issues and examines viral diversity in a critical zoonotic reservoir (bats), at sites of high risk for emergence (wildlife markets) in an EID hotspot (China) (*14*).

SARS and bat-CoVs: Coronaviruses are found in a wide range of animal species (*15*). Before the SARS epidemic, only two human coronaviruses (HCoVs) had been characterized (HCoV-229E and HCoV-OC43) (*16, 17*). Since then three more human coronaviruses (HCoV-NL63, HCoV HKU-1, and MERS-CoV), in addition to SARS-CoV, have been identified in individuals with respiratory infections (*16, 18, 19*). One of these, HCoV-NL63, is thought to be zoonotic and of bat origin (*6*). Our group recently identified a CoV from bats in Bangladesh closely-related and likely ancestral to HCoV-OC43 (*20*) and is currently characterizing CoVs from bats in Saudi Arabia. The animal origins of SARS-CoV were first suspected due to the association among index cases and the trade in wildlife for food (*21*). Initially, civets and other mammals consumed in restaurants in southern China were implicated (*2*), however these species did not exhibit the high seroprevalence and low viral (PCR) prevalence expected from a natural wildlife reservoir of a zoonotic virus (*21*). **In 2004, our group discovered SARS-like (SL) CoVs in free-living wild bat species in China and demonstrated that human SARS-CoV nestled phylogenetically within this group (*4*)**. However, SARS-CoV uses the angiotensin-converting enzyme 2 (ACE2) receptor to gain entry to human cells (*22*), and bat SL-CoVs appeared unable to bind to ACE2. A large number of novel *Alpha-* and *Betacoronaviruses* have since been discovered in Old and New World bats, but few have been isolated (*8, 11-13, 23-27*). In 2012, we isolated and characterized two bat SL-CoVs from *Rhinolophus sinicus* from Yunnan Province, China that use the ACE2 receptor and are closely related to SARS-CoV (**Fig. 1**) (*28*). We found a seasonal shedding pattern for this SL-CoV, with peak prevalence of 30-50%. Bats from this population are hunted for human consumption, posing two crucial questions: **1) What is the risk of these CoVs emerging in humans? 2) Will the conditions that exist in live animal markets in Asia promote further emergence of bat-CoVs in human populations?**

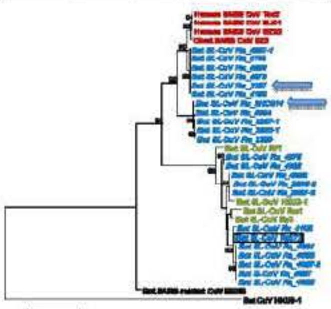


Figure 1. Phylogenetic tree of receptor binding domain sequences of SARS-CoVs (Red), bat SARS-like CoVs discovered by our group in the last 2 years (Blue), and bat SL-CoVs that we published previously in our paper proposing a bat-origin for SARS in 2005 (Green) (*3*). In 2012, we isolated two novel SL-CoVs (SL-CoV-SHC014 and 3367, blue arrows) and have shown for the first time that a bat SL-CoV use the ACE2 receptor which SARS-CoV uses to infect human cells. Unpublished data from Ge *et al.* (in review) (*28*).

Evolution, host-virus co-phylogeny and risk of CoV emergence: There is wide variation in the propensity of viruses for cross-species transmission, within and among viral genera and families (*29*). Coronaviruses undergo genetic recombination by a genomic template-switching mechanism and generate point mutations at a rate similar to that of other RNA viruses, perhaps explaining their capacity for host switching and zoonotic transmission (*15, 30*). This capacity is heightened by the ecology of host species, opportunity for contact, characteristics of the pathogen, and evolutionary (phylogenetic) relationships between hosts (*31-33*). Bats (Order Chiroptera) are the second most diverse group of mammals (~1,200 species) with a wide range ecological and life-history traits that affect their ability to share viruses (*34, 35*) and may explain variation in viral diversity (*36, 37*). Phylogenetic relationships may determine limits to viral binding at receptor sites and to cross-species transmission (*31, 33*), and these factors could be used to predict the risk of spillover (see **Specific Aim 2**). Apart from our own work (see **Section C2b, Fig. 7**), bat and CoV co-evolutionary patterns haven't been rigorously examined. Recent work suggests that most bat-CoV clades correspond to specific bat species or genera (*38, 39*), with little evidence of bat-CoV spillover among species roosting together in the same cave (*40*). There is also evidence for geographically distributed, but related, bat taxa sharing related CoV strains (*8, 38*). In contrast, other studies of wild-caught bats did not find strict co-evolutionary congruence in bat-CoVs for host

species, genera or families (*41-43*). Thus, the same CoV strains may circulate in different bat genera (*41*), and multiple diverse CoV lineages can be found in the same bat species and even individuals (*7, 40, 44, 45*). This, and density of some bat species populations, suggests that viral recombination may be possible in these hosts (*6*). Forced contact in wildlife markets could also facilitate recombination, and may explain divergent Gammacoronavirus strains ancestral to those in birds, in two mammals species in Southern Chinese wetmarkets (*46*). **<u>In this proposal</u>**, we will look for <u>generalizable patterns among bat species and the CoV genotypes they harbor</u>, and use this to <u>examine how phylogeny and contact affect CoV spillover risk</u>.

Host-CoV interactions: an evolutionary approach: The interaction between CoV receptor binding domains (RBDs) and host receptors, e.g. ACE2 for SARS-CoV; dipeptidyl peptidase 4 (DPP4) for MERS-CoV; carcinoembryonic antigen-related cell adhesion molecules (CEACAM) for mouse hepatitis virus; and aminopeptidase N (APN) for hCoV-229E, is critical to understanding limits to host species range (*47-52*). Bats have highly diverse ACE2 receptors at a nucleotide and especially protein level (**Fig. 2**). This is in contrast to other viral receptors in bats, e.g. Ephrin-B2 receptors for henipaviruses (*53, 54*), and DPP4 for MERS-CoV appear to be highly conserved (*51*). Several different genera of bats (e.g. *Myotis*, *Rhinolophus*, and *Rousettus*) have receptors that support viral mediated entry by the SARS-CoV Spike protein (*52, 55*).

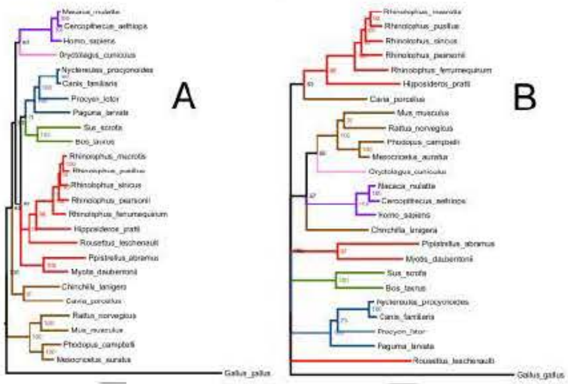


Figure 2. Mammal ACE2 phylogeny using: A) nucleotide data (~2400bp) of ACE2 gene; B) translated protein sequences of same ACE2 genes. All mammal species with available data, including primates (purple), lagomorphs (pink); carnivores (blue); ungulates (green), rodents (brown), and bats (red). Bats are monophyletic and species group with expected taxonomic relationships using nucleotide sequence data (A); but they are paraphyletic a when analyzing protein-level differences (B). This shows functional ACE2 diversity may differ from nucleotide data, and a need to better characterize receptor diversity in a wider range of hosts.

While our preliminary results suggest interesting patterns in bats (**Fig. 2**), the limited number of bat ACE2 sequences precludes robust comparison of co-phylogenetic patterns. **<u>In this study</u>**, we propose to sample dozens of species more than 5 bat families in China, and compare sequence with bats we've sampled globally. This will allow us to build a testable, phylogenetically informed models to examine the extent of, and limits to, batCoV host-range; and will allow us to analyze other receptors like DPP4 for MERS-CoV.

Modeling risk of human infection: The use of mathematical, computational models of viral dynamics has become a standard tool to understand risk of pathogen emergence and spread (*56-60*). However, models that characterize the risk of wildlife-to-human infection require data on contact among populations (*61*), evolutionary constraints of pathogens (*29, 62*), and diversity of novel pathogens (*63*). Because these datasets are usually unavailable, mathematical models can often be theoretical, and of reduced value in predicting risk of pathogen spillover and spread. Building on our group's experience in modeling disease emergence (*64-67*), we will develop a mathematical model that explicitly describes the transmission dynamics and evolutionary dynamics of CoVs in wildlife markets and in bat caves. These models will be parameterized with data we have already collected, and new data from this study, to predict whether novel CoV strains we discover are likely to emerge.

Tests of host range *in vitro*: Receptor usage in different animals is a primary determinant of viral host range. However, while the receptor and receptor binding domains (RBDs) of human-infecting CoVs have been studied intensively, bat-CoVs have not (*22, 47*). In this study, we will determine the RBD of bat-CoVs, develop pseudovirus assays (*68*), and work with a humanized mouse model expressing ACE2 receptor. This provides a way to experimentally test hypotheses on the host-range of novel coronaviruses, even from sequence data. However, despite a plethora of novel CoVs in the recent literature (*38, 39, 44, 45*), there has been little work towards this goal. Furthermore, the recent discovery of MERS-CoV, which uses DPP4, and the use of other receptors for other CoVs (*69*) suggest that this work will be highly significant for other CoVs.

B. INNOVATION:

This project is an innovative fusion of virology, ecology, and mathematical modeling. The analysis of CoV genetic diversity in bats and other mammals in southern China, combined with characterization of and co-phylogenetic analysis with CoV functional genes (e. g. ACE2, receptor of SARS-CoV and DPP4 for MERS-CoV) has not yet been attempted, and **<u>will allow us to better understand the patterns of host-switching</u>**. Previous studies using molecular clock analysis have found that the bat SARS-like-CoV to civet/human SARS-CoV divergence

ranged from 7-17 years (mean 4.9) before the 2003 outbreak (*7, 70*). We will use a novel **phylogenetic and mathematical modeling approach to examine how dynamics of contact and pathogen transmission among hosts in markets drives viral evolution and emergence**. We will determine **how many years it takes for a coronavirus to evolve an R_0 >1 and therefore have epidemic potential** using a modeling framework that combines evolutionary changes and multi-host dynamics (Specific Aim 3), expanding on published approaches (*71-73*). We will then **simulate coronavirus emergence under different market conditions to identify most likely scenarios that can inform strategies to prevent future outbreaks.** Finally, we will use pseudovirus binding assays, *in vitro* infections and humanized mice expressing ACE2 to test our analyses on the novel viruses we have, and will, identify.

We will use our multidisciplinary approach to examine fundamental questions on how the wildlife trade, wetmarkets and other target interfaces promote the emergence of coronaviruses, and **assess the risk of future spillover of CoVs from bats or other mammals and humans**. In particular, despite 10 years since the emergence of SARS and the discovery of 60+ novel bat-CoVs, three significant issues remain unanswered: **1) What are the natural limits to CoV host range, and can this be predicted by the host-receptor-virus relationship; 2)** Are the conditions in wildlife markets sufficient to allow enough interspecies transmission that coronaviruses **can evolve the ability to infect new hosts, including humans**, either by accumulation of point mutations, or by recombination events; or **3)** Is the expansion of the wildlife trade **bringing expanded diversity of CoVs into the enhanced human-animal interface present in wet markets**?

C. APPROACH

C1: Specific Aim 1. Assessment of CoV spillover potential at high risk human-wildlife interfaces:

C1a) General strategy and supporting studies: SARS-CoV emerged in live animal markets in Guangdong, with unrelated spillover events in at least five of seven municipalities, suggesting widespread introduction into wildlife markets within this city (*21*). We propose to characterize the species composition of bats and small mammals in wildlife markets where there is a high degree of contact between animals and people. We will identify additional high risk interfaces that may occur in southern China such as guano collection, which we have recently identified as a potential CoV exposure risk in Thailand (*12*). We will interview people at high-risk interfaces and who are enrolled in acute respiratory or influenza-like illness surveillance programs conducted by our colleagues at CDCs in Shanghai, Guangdong, Yunnan, and Guangxi. These data will be used to parameterize the contact process (χ) in our mathematical model of CoV emergence (**see Aim 3**).

We will assess **1)** whether market conditions provide enhanced capacity (increased **evolutionary opportunity**) for bat-CoVs to evolve the ability to infect other hosts, either via repeated inter-species transmission, positive selection or recombination events; and **2)** whether the intake of wildlife from Southeast Asia by China introduces a greater diversity of hosts and a correspondingly diverse group of CoVs (increased **ecological opportunity**). We will conduct CoV pathogen discovery in samples from humans and wildlife at these sites and examine their receptor binding domains to identify their ability to bind to ACE2, DPP4, or CEACAM receptors in humans. We will compare CoV diversity in China with that in wildlife across Southeast Asia (from our current work on other funded programs, and published data) that may potentially enter China's wildlife trade. Data from this aim will be used to assess the likelihood of inter-species bat-CoV transmission (see also **Specific Aim 2**).

Working in high-contact human-wildlife interfaces can be challenging. However, we have already collected significant preliminary data to accomplish Aim 1. We have located and surveyed wildlife markets in Yunnan, Guangdong, GuangXi and Fujian provinces, and have identified populations that hunt and consume bats in Yunnan province. We have begun to characterize the species composition of free-ranging bat populations and have collected samples **from over 1000 bat individuals (28 spp.) from 35 localities in over 15 (two-thirds of all) Chinese Provinces**. We will also utilize archived wild bat, rodent, and civet samples collected by our team in Malaysia, Thailand and Indonesia on another large federally-funded project to provide samples of species regularly imported into China (**section C1b**) (*21, 74*).

Wildlife Markets: Ten years following the SARS-CoV outbreaks, there is little information available on the *current* diversity of bats and other mammals available in the wet markets in southern China. One study found that 91 species of vertebrates, including 40 mammal species, were being traded in Guangxi, China (*75*). Further, little data is available on the origin of wild animals brought into the market system. In some cases, animals may be locally collected, while in other cases animals may be imported from Southeast Asia, including adjacent Vietnam (*74-76*) – factors which will affect the diversity of CoVs. Captive and free-ranging rodents are found in markets and may be an additional host for CoVs (*77*). We have worked with Yunnan Institute of Endemic Diseases Control and Prevention since June 2012 (see **Letters of Support**). We have conducted initial surveillance in Nujiang, Baoshan Denong and Xishuangbanna prefectures and Ruili, which is a major wildlife trade gateway between Myanmar and China **(Fig. 3)**. We have collected 187 small mammals from markets in Yunnan and tested them for coronaviruses using a 1-step PCR assay (*78*), finding 2/21 shrews (*Crocidura attentuata*) are CoV-positive.



Figure 3: Map of wildlife trade routes from Southeast Asia into China. Modified from (*79*).

Other animal samples available for this project: To date, our group has collected more than 90,000 high quality specimens from 15,000 animals representing key wildlife reservoirs for zoonoses such as bats, rodents and primates under our USAID-EPT PREDICT project. Clinical samples include blood, throat swabs, feces and urogenital swabs and represent animals from 10 different countries including Bangladesh, India, Malaysia, Thailand, Indonesia, China, Brazil, Bolivia, Colombia, Peru, and Mexico. 50,000 of these samples originate from Asia, and are currently being screened for novel coronaviruses (**See Section C2a, Fig. 6**). **We have also collected more than 500 bat specimens representing seven species from the Kingdom of Saudi Arabia in collaboration with Saudi Arabia's Ministry of Health and Columbia University.** Nearly 20,000 of our samples come from bats, and will be used to analyze CoV diversity along with novel CoVs we identify.

Identifying novel CoVs in wild bats in China: We have already conducted significant CoV surveillance in China for bats, other wildlife and humans. For this, we use pan-coronavirus PCR protocols based on conserved RNA-dependent RNA polymerase (RdRp) motifs A and C to screen samples at Wuhan Institute of Virology (*80*). Besides a large number of SL-CoVs, we have detected several novel bat-CoVs including strains closely related to CoV HKU4/5, CoV 1A &1B, CoV HKU 2, 6, & 8. For the first time, we have also isolated and characterized a bat-CoV from China that uses ACE2 receptors (see **Section C3a preliminary data**) (*28*). **In all, we have identified sequences from 268 novel bat-CoVs (140 from China alone) from bat species collected in Bangladesh, Thailand, Mexico, Brazil and China (See Section C2a, Fig. 6).** We have an additional 5,000+ clinical samples from free-ranging bats and rodents from Guangdong province, from an ongoing study which are being screened for viral pathogens, including CoVs at Guangdong Entomological Institute.

Survey of people highly exposed to wildlife in Guangdong, China: We have worked with Guangdong CDC since 2008, under a currently active IRB protocol, to interview and sample people working in live animal markets, hunters and restaurant workers with a high level of exposure to animals. We have interviewed volunteer participants about the nature and frequency of animal interactions; collected biological samples (blood, feces, sputum), and trained participants to collect animal blood samples (dried blood spots on filter paper) from animals they butchered or hunted. We enrolled 1300 participants across 12 sites within Guangdong Province (**Fig. 4**).



Figure 4: Sites of current human sample collection by Guangdong CDC for zoonotic pathogen surveillance in Guangdong Province, Southern China. Each star represents a large wildlife market where we have enrolled market and restaurant workers (total = 1,300) for our zoonotic pathogen spillover study. Seventeen people had IgG antibodies to SARS-CoV and a follow-up study is underway.

Samples have been tested for antibodies to animal pathogens, including SARS-CoV. **Of the 1300 serum samples screened using a SARS-CoV ELISA, 17 were positive for IgG antibodies to SARS-CoV.** These patients were not acutely ill at the time of sample collection, and this finding suggests one of three possibilities: 1) that SARS-CoV is still circulating in Guangdong markets; 2) that these people may have been exposed during the time of the 2002-3 outbreak; or 3) that the ELISA used is cross-reacting to another CoV. Review of their history of wildlife exposure is currently underway. In Shanghai, the Shanghai Municipal Center for Disease Control and Prevention (see **Letters of Support**) currently conducts surveillance on people with influenza-like illness in rural communities surrounding Shanghai. We will develop a similar study of people in

these communities who have exposure to wildlife. We will review and re-screen archived blood samples at Guangdong CDC for other bat coronaviruses once we determine candidates that could likely infect humans. to see whether there is exposure to CoVs other than SARS. We will re-screen these samples with specific serological assays based on bat-CoVs that will help differentiate between SARS-CoV IgG and other bat-CoV IgG to see whether there is exposure to CoVs other than SARS (*3, 81*). We will expand our survey to Guangxi, Fujian, Shanghai and Yunnan provinces to survey regions where SARS-CoV was not reported, but where wildlife trade, hunting, and bat guano collection is common.

C1b) Market characterization, wildlife sampling and human surveys: We have conducted surveillance at the wildlife markets of Guangdong where early cases of SARS-CoV were identified. From 2011-2013 we interviewed and sampled animal vendors, hunters and restaurant workers who butcher wildlife (**See Section C1a, Fig. 4**). For this proposed study, we will identify 10 markets in Guangxi, Yunnan, and Fujian Provinces (**Fig. 5**). We will characterize the physical size, number of vendors, diversity and abundance of mammalian species in each market. A questionnaire will be developed based on the one we used in Guangdong, to collect data on the nature and frequency of animal exposure of people who work in markets or hunt wildlife. We will conduct interviews to determine which bat species are sold, typical numbers, and source locations. We will collect information about recent acute respiratory illness and include those who have had undiagnosed acute respiratory symptoms within 3 months of the survey. We will then screen volunteers from this cohort for bat-CoV antibodies using existing and newly developed assays. We will compare exposure rates between people who are highly exposed to wildlife and a control group from the same regions.



Figure 5: Proposed sampling sites in Southern China (Guangdong, Guangxi, and Fujian Provinces) for the current study. Arrows indicate wildlife trade routes. Letters indicate wild animal markets in Guangdong (A-R), Guangxi (S-W), Hunan (X) and Fujian (Y).

In Shanghai, where wildlife markets are less common than southern provinces, we will interview voluntary participants under surveillance by Shanghai CDC for influenza-like illness. We will compare CoV exposure rates in people with acute respiratory illness to a control group from the same region (**see letter of support**).

Wildlife sampling: We will locate wild bat populations used to supply local markets in Yunnan, Guangdong, Guangxi, and Fujian. We will sample a minimum of 30 individuals from 30 different bat species representing but not limited to the following families: *Rhinolophidae, Hipposideridae, Vespertillionidae, Mollossidae,* and *Pteropodidae*, all of which are known to carry *alpha-* or *betacoronaviruses* and are consumed by people (*4, 7, 82*). Bat SL-CoV PCR prevalence is 10%-38% (*4, 24*). Given 10% prevalence in bat populations, sampling 30 individuals would ensure a CoV detection probability of 95%. In all wildlife markets, we will opportunistically sample a variety of insectivorous and frugivorous bats, and other mammals if available, taking fresh feces or rectal swabs, saliva (oropharyngeal swab), and blood. A small number of bats will be sacrificed as vouchers and to collect intestinal tissue for CoV receptor analyses if required. We will use cyt-b to identify host species.

Human exposure to CoVs study: Expanding on our work in Guangdong, we will develop a voluntary study of animal vendors and hunters in Guangxi, Yunnan, and Fujian provinces in cooperation with local Bureaus of Public Health and CDCs. We will develop a survey to identify people with high exposure to wildlife, particularly bats, and will recruit volunteers, collect blood, sputum, and stool sample from each enrolled participant. We will screen sera for antibodies to SARS-CoV, other alpha & beta coronaviruses including MERS-CoV, and novel bat-CoVs. We will screen stool from CoV seropositive participants for CoV nucleic acid. We will also develop specific bat-CoV serological assays and share these with our Chinese collaborators. In each province in southern China we will aim to include 10 markets and survey 20 vendors per market; 20 additional wildlife hunters per province (220 case subjects); 400 control subjects from the general population near the markets in each province (total of 620 people per province). For Shanghai, we will enroll 200 acute respiratory illness cases and 400 non-respiratory controls (600 total), The total number of human subjects will be 2460. The study will be conducted in Guangxi, Yunnan, Fujian and Shanghai provinces (see **Section E, Human Subjects**).

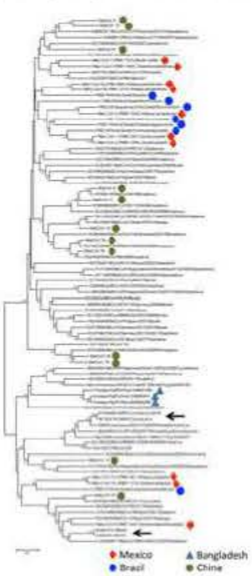
C1c) Data analysis: Human sera and stool samples will be tested at provincial CDC labs (see **letters of support**) and animal samples will be screened at the Wuhan Institute of Virology (Co-I, Shi)**.** Serum or plasma samples will be tested for CoV antibodies using ELISAs specific for SARS-CoV and bat SL-CoVs that we have developed (*4, 68, 83*). Fecal and saliva samples will be tested for CoV viral nucleic acid using a series of pancoronavirus PCR assays that target a region in the RdRp that is highly conserved among coronaviruses and for which we have a positive control, developed by our group under another federally-funded contract (*13, 23,*

84). The RdRp gene will be sequenced from all positive PCR samples and used to build co-phylogenetic trees (see **Specific Aim 2**). We will also test these pathogens for recombination events in markets vs. wild sampled CoVs after viral strains are characterized. Data from **Aim 1** will be used to parameterize mathematical models of viral transmission (**Specific Aim 3**) in markets to estimate relative risk of emergence depending on different diversity of mammals, contact rates, size of markets, and evidence for human exposure to bat-CoVs.

C1d) Potential Pitfalls and Solutions: We may find lower than expected levels of wildlife diversity in markets in Southern China. If this occurs, we have access to tens of thousands of wildlife samples from over 20 countries globally from work on a current NIAID R01 (Daszak PI) to assess diversity of viral pathogens in bats in Asia and Latin America, a large multi-year contract from USAID (Emerging Pandemic Threats: PREDICT program, Daszak PI) to conduct surveillance and pathogen discovery in wildlife in Asia and Latin America and two Nipah virus R01s. We have already discovered >250 novel CoVs from bats in these countries (**Section C2a**) including >100 from China. A second setback would be that access to markets becomes restricted due to political sensitivities. We are working closely with long-term local collaborators at ECNU and the Institute of Virology, Wuhan, both of which institutions are well respected nationally. The Institute of Virology is the National Center of Excellence for viral pathogens, and has Federal authority for viral research. Furthermore, we have shown through our work with Guangdong CDC that we can conduct long-term collaborations in these sites. Finally, we have selected a large number of wildlife market sites, so the closing of one will not affect all sampling activities.

C2: Specific Aim 2. Receptor evolution, host range and predictive modeling of bat-CoV emergence risk:

C2a) General strategy and supporting studies: *Can we use information on CoV sequence, host sequence and behavioral traits and population dynamics at critical human-wildlife interfaces to predict which CoVs are most likely to emerge?* To answer this, we will use data from our characterization of bat-CoVs, host range, receptor genes, serological data, and from field-collected data to build and parameterize three related models. **First**, using phylogenetic reconciliation we will map the co-phylogenetic patterns of bats and their CoVs using neutral and functional markers (RBDs and host receptor genes). We will compare free-ranging and market-sampled species assemblages and test the related hypothesis that wildlife markets disrupt 'natural' bat – CoV host associations and increase recombination and/or accelerated evolution to facilitate emergence. **Second**, we will construct generalized linear models that encompass phylogenetic information to test the two related hypothesis that spillover potential and host-range of bat-CoVs is limited by: 1) opportunity for contact; or 2) phylogenetic relatedness of host species and their receptor genes. **Third**, we will use mathematical matrix modeling to investigate bat-CoV transmission and evolutionary dynamics, and test the potential of novel CoVs to infect humans, bats, and other market animals. This model will be informed by serological data, market surveys, and receptor binding data from bat cell line and humanized mouse inoculation studies.



Phylogenetic studies of known and novel bat-CoVs: Phylogenetic methods can be used to identify recent host shifts and spillover events of CoVs, often these events are due to anthropogenic changes to host ecology, e.g. *Rhinolophus* spp. and human/civet SARS-CoV in the wildlife trade (*4, 7*). It has been proposed that repeated passage between civets and humans in wet markets facilitated SARS-CoV evolution towards greater human and civet ACE2 receptor affinity (*85*), and accelerated evolution and positive selection in CoVs was detected after host spillover (*86*). It is not known if bat-CoVs follow predictable patterns of co-phylogeny between host and virus; many studies found unique CoV strains circulating in different bat lineages, but also multiple CoV strains have been identified in the same bat species and individuals (*7, 40, 44, 45*).

Figure 6 (above): Phylogenetic tree (RdRp) of selected bat-CoVs from Genbank, including as subset of the 268 novel bat-CoVs discovered by our group through our USAID-EPT PREDICT pathogen discovery work in China, Brazil, Bangladesh and Mexico.

Wildlife trade and market dynamics may promote the cross-species transmission of distinct bat-CoV strains and facilitate viral recombination within these hosts (*46*); the extent of this will depend on the role of host phylogeny vs. contact in limiting bat-CoV spillover. Using our extensive database of bat and other wild animal CoVs that we have characterized, isolated, or are available on Genbank, we will examine these constraints for known and novel CoVs we identify. Over the past four years, we have conducted large surveys of bat pathogens globally, including the discovery of sequences from 268 novel bat-CoVs (including 140 from China) (**Fig. 6**).

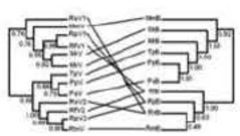


Figure 7 (left): Host-pathogen co-phylogeny of bat-CoVs from China (*43*). Bat genera: R, *Rhinolophus*; Mm *Miniopterus*; Mr, *Myotis*; P, *Pipistrellus*; V= viral sequence, B= bat sequence. This figure suggests rhinolophid CoVs may have a greater ability to jump hosts. Warrants further investigation using functional genetic markers and data from more species.

C2b) Co-phylogenetic analysis of bat-CoVs: We will use coronavirus and host sequence data generated in this project, from archived samples that we collected from bats just after the SARS outbreak, and previously published CoV strains from a diverse range of host species to quantify co-evolutionary patterns and host range in bat-CoVs. Combined analyses of host and viral phylogenies **will allow us better understand if host phylogeny (and receptor gene similiarity) can predict CoV host switching and whether or not market systems have disrupted the "natural" patterns of CoV association** (e.g. **Fig. 7,** from (*43*)). We will reconstruct phylogenetic relationships of CoVs using a combination of the HEL, N, RdRp, and S genes, as each has a different evolutionary rate and will allow us to test patterns of cophylogeny at different taxonomic scales. We will reconstruct host species relationships from tissue collected in our study using both neutral (mitochondrial and nuclear, e.g. cytB and RAG2) and functional (e.g. ACE2 CoV receptor) host genetic markers. Multiple alignments will be performed MAFFT (*87*), and phylogenies estimated using maximum likelihood (*88*) and Bayesian inference (*89*) for each viral and host gene, and concatenated virus datasets when no viral recombination is detected. We will test for statistical significance using ParaFit implemented in CopyCat (*90*) and AxPcoords (*91*), and visualize these using TreeMap v2.02β (*92*). These methods will allow us to identify which particular host-virus associations contribute most to the observed patterns. We will partition our dataset by collection localities and higher-level taxonomic groups to test co-phylogenetic significance at multiple spatial and taxonomic scales. To test the null hypothesis that there is no pattern of co-evolution we will perform permutations to randomized hosts–virus associations and then measure congruence relative to the host tree. By comparing the patterns of host-CoV co-phylogeny in natural bat communities (cave sites) vs. wet markets, we will be able to identify anomalies that may likely signal recent spillover events. To test for genetic recombination in market vs. wild-collected bat-CoVs, we will use sliding window analysis (*7*) and RDP3 v3.44 software (*93*). We will use previous methods to test for positive selection and identify specific virus residues under selective pressure (*94*).

Quantifying CoV strain sharing between host species: We will use viral sequence data from RdRp and S genes to delimit unique CoV "species" or "genotypes" at different taxonomic and sampling levels. We will test for non-random patterns of association of viral community assemblages between species (*95-97*) (*98*). This will involve calculating Jaccard's index of similarity (J) for the viral assemblages between pairs of species and testing for deviations from that expected by random chance using Monte Carlo randomizations (*99*). Deviation from the null model will be calculated as the difference between the mean J observed (J_{obs}) in the data and the mean J expected, such that $J_{dev} = J_{obs} - J_{null}$. Positive values of J_{dev} will thus indicate that CoV community assemblages between host species are more similar than would be expected by random chance, while negative values would indicate greater dissimilarity in the viral assemblages than would be expected by chance.

C2c) Predictive model of CoV host-range and diversity: We will develop a predictive model of host-range for bat-CoVs using data of bat distribution in natural caves and the markets, geographic ranges, ecological and behavioral characteristics of host species from our field studies and the literature, host and viral phylogenies, and associations of host species to particular CoV strains/clades. We will include phylogenetic distance between bat species and other mammal hosts from various neutral and receptor genes generated in this study. We will use CoV similarity indices (Jaccard, above) as our response variables in multiple regression models, i.e. generalized linear models (GLMs) and phylogenetic generalized linear mixed models (PGLMMs) with relevant bat ecological, phylogenetic, morphological, behavioral, and life history traits as our predictor variables, to assess the relative contribution of host phylogeny, viral traits, or species-specific ecological traits in explaining CoV diversification and sharing. We will calculate indices of host specificity that account for host phylogeny (*100, 101*), to further test hypotheses of whether bat-CoVs are more likely shared between host ecological groups or among species with similar life-history traits vs. relatedness. All statistical analyses will be conducted in R with relevant packages for community ecology and species diversity (vegan, fossil), and phylogenetic modeling (ade4, ape).

Extension of this model beyond China will allow us to map **a global spatial and phylogenetic risk gradient for CoV emergence based on host species traits, mammalian phylogeny (including functional CoV receptor genes), and relatedness of CoVs**. Further, we can use the results from our logistic regressions to identify gaps in surveillance, where bat species are found to share a lower than expected number of CoV strains given a threshold level of contact and relatedness with other host species. We will test our predictions of host range from the analytical model for bat-CoVs using synthetic reconstruction of bat-CoVs and *in vitro* studies of ortholog

receptor binding with different mammalian cell lines (**Aim 3**). Specifically, we will evaluate the ability of novel bat-CoVs to recognize and bind to selected receptors (ACE2, CEACAM, APN, receptor for alpha-CoV, or DPP4/CD26, receptor of MERS-CoV) reconstructed from divergent bat taxa. We envision an iterative process over the first few years of this grant whereby initial data are generated from known host-CoV associations, results from the model will be tested experimentally, and then data from experimental studies will be used to refine the models and better inform field sampling in China and globally.

Analyses of literature database: We have built a database of virus-host associations for 131 bat species and all 50 unique ICTV recognized bat viruses. We used a logistic GLM regression approach with host and virus variables, and found that host phylogeny (i.e. phylogenetic distance to other bat host species) was a strong predictor of observed virus sharing across bat species (trend with phylogeny only shown in **Fig 8**). We will adapt this approach by using host genetic distance of functional receptor genes instead of neutral markers, and CoV data collected from our standardized survey efforts.

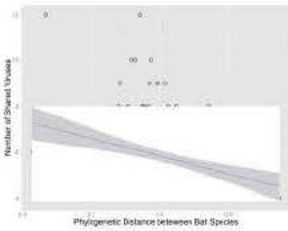


Fig 8. Scatterplot showing a decrease in the number of shared viruses with decreasing phylogenetic relatedness among bat species. Dataset includes all bat species pairs with >3 shared viruses for ~200 bat-virus associations form the literature (Olival, unpublished). Pairwise phylogenetic distance from maximum likelihood tree using cytochrome B mtDNA data.

C2d) Modeling the dynamics of CoV spillover risk: A key question in EID research is the role of viral evolution in enabling pathogen emergence. While some EID pathogens cause epidemic or pandemic disease because they readily transmit among humans ($R_{0,Human} > 1$, e.g., HIV, A/H1N1pdm), or only spillover directly from animals ($R_{0,Human} = 0$, e.g. West Nile Virus). Others, including MERS-CoV, may spillover regularly to humans, and even cause small clusters of human-to-human transmission, but have not yet caused a major epidemic or pandemic ($1 > R_{0,Human} > 0$, e.g., Nipah virus, monkeypox, Influenza H5N1). A looming issue is the likelihood of such a pathogen evolving to become a major epidemic or pandemic (i.e., $R_{0,Human} > 1$). Divergence times between ancestral bat-CoVs and hCoVs can vary widely and provide a timeline of past spillover events, e.g. 560+ years between hCoV-NL63 and its progenitor alpha-CoV (*6*) and ~20 years between bat SARS-like CoVs and human or civet SARS-CoV (*7, 70*).

The limits on SARS emergence are still unclear: Were the bat SL-CoVs unable initially to bind to human receptors, or was it necessary for a precursor CoV to evolve and adapt to humans for SARS-CoV to emerge? Were civets or similar non-bat, non-human hosts a critical intermediate evolutionary step in the transition from bats to humans, or were they incidentally infected along with humans simply by virtue of similar receptors? To examine the timeline for different emergence pathways, we have built a model framework (below) to represent the wildlife market environment and include viral ecology and evolution. We will use a matrix framework (*72, 102*) to determine how the pathogen is transmitted among different host species and between locations. We have already built the framework of this model (below), and have listed the data that we will collect in the current study to parameterize it (**Table 1, below**). To incorporate strain variation and evolution, we will adapt the approach of Antia *et al.* (*71*) by integrating a branching process approach to our matrix framework. **We will use these techniques to develop "What If" scenarios that predict how different strains of CoV would emerge, and potentially evolve, in different market systems within Asia and elsewhere** (e.g. scenarios with different host diversity and different levels of host-host and host-human contact within markets).

To examine strain evolution, we will model ***n*** possible strains, where strain 1 is the initial variant, and strain ***n*** is the variant that has a human $\mathbf{R_0} > 1$, with ***n-2*** variants in between, which may each have their own $\mathbf{R_0}$ that depends on the host community using 'Who-Acquires-Infection-From-Whom' (WAIFW) matrix framework (below). Following Antia *et al.* (*71*), we assume the mutation rate, μ, is the same for all variants, that only single mutations can occur, and we ignore back-mutation. However, we will reconsider these assumptions if changes in these can alter the expected outcomes of the mathematical results. We also assume that the total number of secondary infections generated by an individual with variant ***i*** is Poisson distributed with mean $\mathbf{R_0^{(i)}}$. A proportion μ of the variants will have mutated or recombined into type ***i+1***, while the proportion $(1-\mu)$ remains the same, as type ***i***. We will separate out the cases of mutation and recombination by placing different restrictions on the changes that could occur in the strains as they move from type ***i*** to type ***i+1***. These assumptions result in the probability generating functions:

$$f_i(s_1, s_2, \ldots, s_m) = \exp\big(-(1-\mu)R_0^{(i)}(1-s_i)\big)\exp\big(-\mu R_0^{(i)}(1-s_{i+1})\big) \quad \text{for} \quad i < m$$

(5) $$\text{otherwise} \quad f_m(s_1, s_2, \ldots, s_m) = \exp\big(-R_0^{(m)}(1-s_m)\big)$$

Through this branching process approach we can gain insight into the limitations and possibilities that stochastic processes may impose on the evolution of strain diversity in both limited diversity settings (e.g., only bats and humans), and highly diverse environments (e.g., markets with other hosts such as civets and bamboo rats). We can also adapt this methodology to compare mutation, which we expect to take small incremental movements in a fitness landscape that may have low fitness valleys between a wild-host adapted strain and a human or other host adapted strain, and recombination which may be able to take larger leaps across a given fitness landscape. Using this framework we can vary the $\mathbf{R_0}^{(i)}$ depending on the fitness of the mutants in various hosts, and the host diversity and abundance, simulating the complex fitness landscapes of real CoV systems. We can calculate the number of secondary hosts infected as $\mathbf{R_0} = \chi\phi\tau$, where τ is the duration of infectiousness, and χ is the rate of contact. Our receptor binding studies and predictive GLM models of host range will be used to inform ϕ, the joint probability that a susceptible host becomes infected when exposed. We will model our system both mathematically from a simple $\mathbf{R_0}$ perspective for insight, as well as using a spatial stochastic-birth-death simulation implementation to understand the implications of multiple scales of variation, including mutation and recombination and the implications for stochasticity for CoV emergence. To do this we will expand our basic equation, $\mathbf{R_0} = \chi\phi\tau$, into a matrix formulation to incorporate the multiple hosts within this system. Each strain and spatial location (e.g., market), can be represented by a different matrix. Thus we have:

$$X_k = \begin{bmatrix} \chi_{1,1,k} & \cdot & \cdot \\ \cdot & \cdot & \cdot \\ \cdot & \cdot & \chi_{i,j,k} \end{bmatrix} \qquad \Phi_k = \begin{bmatrix} \phi_{1,1,k} & \cdot & \cdot \\ \cdot & \cdot & \cdot \\ \cdot & \cdot & \phi_{i,j,k} \end{bmatrix} \qquad T_k = \begin{bmatrix} \tau_{1,1,k} & \cdot & \cdot \\ \cdot & \cdot & \cdot \\ \cdot & \cdot & \tau_{i,j,k} \end{bmatrix}$$

(1)

which we can use to define a 'WAIFW' Ω_k matrix (*73, 103*) of which the eigenvalue gives us an estimate of $\mathbf{R_0}$ for the whole system, for a given strain and location. The 'WAIFW' matrix is:

$$\Omega_k = \begin{bmatrix} \chi_{1,1,k}\phi_{1,1,k}\tau_{1,1,k} & \cdot & \cdot \\ \cdot & \cdot & \cdot \\ \cdot & \cdot & \chi_{i,j,k}\phi_{i,j,k}\tau_{i,j,k} \end{bmatrix}$$

(2)

Critically, this enables us to analyze certain 'what-if' scenarios. For example, we can examine the role of civets in emergence by assuming that the strain which initially infected civets had to evolve in order to then infect humans. This would give us two strains in a single location, each with its own $\mathbf{R_0}$. Alternatively, we can assume that all three SARS-CoV host species (bats, civets, humans) were in the same market place, and a single CoV strain. In this case we would have a single matrix, with all three species, and values in every cell of the matrix. By keeping the separate pieces of the $\mathbf{R_0} = \chi\phi\tau$ equation, in the matrix form, we can examine potential public health control measures (e.g. quarantine, culling or separating species into different market locations) (*104*), which might also vary depending on the nature of receptor binding and strain evolution. To account for assumptions, we will investigate the implications of mixing in a stochastic environment. We have already built a stochastic-birth death, discrete event simulation of the spread of EIDs for Avian Influenza in multi-species markets and farms. We will adapt these simulations for strain and receptor diversity interactions with multiple species of CoV hosts. **This suite of modeling approaches will allow us to integrate our ecological and molecular approaches to understanding the potential pandemic emergence threat posed by the whole suite of bat-CoVs.**

Table 1: Data Needs for Model:

Parameter	Description	Sources
τ_{Human}, τ_{Bat}, $\tau_{\text{Other?}}$	Duration of infectiousness, Humans, Bats, other spp.	Humans (*57, 105-108*), Bats (*7*), other species (*108, 109*)
$\phi_{\text{Human->Human}}$	Joint probability an infected Human can transmit to susceptible Human	(*57, 105-107*)
$\phi_{\text{Bat->Human}}$, $\phi_{\text{Other->Human}}$ $\phi_{\text{Bat->Other}}$, $\phi_{\text{Bat->Human}}$ $\phi_{\text{Other->Other}}$	Joint probability an infected host can transmit to susceptible; can use receptor binding in host species for parameterization	*(*109*)
$\phi_?$	As above	Generally assume 0 or $\phi_{i,j} = \phi_{j,i}$ *
$\chi_{i,j}$	Contact rates	Market Surveys, using map overlap for non-market areas.
μ	Mutation rate	Literature
ξ	Recombination rate	Literature
N_{Human}, N_{bat}, N_{other}	Population density of bats, humans, other	Market surveys, census & transect data.

* Use knowledge of receptor bindings to appropriately upscale or downscale relative to human-to-human case of SARS and laboratory studies on other animals. We will run sensitivity analyses for these parameters.

We will assume that twice the estimate for SARS $R_{0,Human}$ rounded up to the max of the 95% CI to give 5 or 10 represents a near maximum, and 0 forms a lower boundary. We will assume τ is constant regardless of species and again do sensitivity analysis using SARS-CoV values. We test the following hypotheses: 1) That recombination can either substantially boost (H_A: $\Delta Pr > 0$) or mutation have the same effect (H_A: $\Delta Pr > 0$) on the probability of CoV spillover into humans, or that only recombination and mutation together provide a substantial boost to spillover probability (H_A: $\Delta Pr > 0$); 2) That known (e.g. civets) or unknown intermediate animal hosts or no intermediate hosts are necessary for CoV spillover to humans; 3) That high diversity of intermediate hosts either increases or decreases the probability of CoV spillover into humans. We will use our modeling framework to examine the potential CoV spillover in different markets, using the market data from Specific Aim 1, evolutionary characteristics of the CoVs from Aim 2, and specifically-acquired data to parameterize the model. **Table 1 (above)** lists parameters in the model, and gives available sources for data.

Previous experience of modeling disease emergence: Our group has used mathematical models to test hypotheses on zoonotic disease emergence for over 15 years. We use computational models that are tailored for the specific pathogen type or combination of hosts involved, and parameterize these with extremely detailed datasets specific for the emergence event. We then run simulations to test hypotheses on the spillover of viruses and the emergence of zoonoses. For Nipah virus (NiV), another bat-borne zoonosis, we obtained data from pig production facilities in Malaysia (*110, 111*), from experimental infection of bats and *in vitro* under BSL-4 conditions for viral transmission parameters (*112, 113*). We used this approach to demonstrate the cause of NiV emergence (*111*). We have successfully used similar approaches to demonstrate viable causal mechanisms for the emergence of Hendra virus (*114*), Avian influenza (*115-117*) and West Nile virus (*118-120*).

C2e) Potential pitfalls and solutions: The diversity of coronaviruses that we identify may be inadequate for robust co-phylogenetic analysis. We have already shown proof of concept in preliminary data through USAID and NIAID funded projects that we have detected new coronaviruses in most bat species examined; there has been a large amount of research from several groups showing a broad diversity of coronaviruses; previous studies from us and other groups have provided evidence of a diversity of coronaviruses associated with bats and there is high likelihood that we will identify more. In China specifically, 23% of bat samples we have screened were positive for CoVs, thus we do not anticipate a lack of diverse CoVs (*28*). For modeling studies, not all necessary parameters may be easily obtained. We will use information from the SARS-CoV outbreak, where we have detailed data from the WHO investigations on serology and viral isolation from market wildlife; and from our recent and current work in Guangdong province; and an ongoing study on avian influenza in Shanghai and Guangdong markets (Co-I Zhang). Lastly, for parameters that we cannot actually estimate, we may be able to posit reasonable limits. For example we can constrain the probability of spillover: it must be greater than 0, since SARS did in fact spillover (*106*), but it is very unlikely that this probability is higher than the within species transmission probability. If the rate of transmission within a host species is unestimatable, we can use data from other diseases in similar species, such as bat rabies. Thus we can readily perform a sensitivity analysis for unknown parameters within a range that is biologically plausible, using sensible constraints.

C3: Specific Aim 3. Testing predictions on CoV inter-species transmission:

How can we test predictive strategies to understand which viruses have the capacity to 'jump hosts'? To answer this, we will analyze the interspecies infection or transmission of CoVs we have identified, particularly the SARS-like CoVs and CoV HUK4/5 that is closely related to MERS-CoV (hCoV-EMC) from Saudi Arabia. Our main approach will be: 1) *in vitro* infection experiments using pseudoviruses carrying the spike proteins (wild type or mutants) or live viruses in cell lines of different origins; 2) binding affinity assays between the spike proteins (wild type or mutants) and different cellular receptor molecules; and 3) humanized mouse experiments if viruses are identified of significant human infection potential (see **Ralph Baric, Letter of Support**).

C3a) General strategy and supporting studies: We will sequence the spike (or other receptor binding/fusion) protein genes from all bat-CoVs we identify, creating mutants of these to identify how significantly each would need to evolve to use ACE2 or CD26/DPP4 (receptor for MERS). We will then use receptor-mutant pseudovirus binding assays, *in vitro* studies with a wide range of cell lines from bats, other mammals including primates and human cell lines, and with humanized mice where particularly interesting viruses are identified phylogenetically, or isolated (see **Ralph Baric, Letter of Support**). These tests will provide direct public health-relevant data, and also iteratively improve our predictive model to better target bat species and CoVs during our field studies to obtain bat-CoV strains of the greatest interest for understanding the mechanisms of cross-species transmission.

Experience working with receptor mutants & pseudovirus binding assays: We have established a stable pseudovirus assay for SARS-CoV and SARS-like CoV and tested the infectivity of these spike proteins in cells expressing ACE2 from human, civet and bats (*52, 68*). We have demonstrated that several bat species are susceptible to the SARS-CoV and that some SARS-like CoV strains can use human ACE2 for cellular entry (*52*). Minor mutations in S proteins or ACE2s greatly affected the receptor binding and finally abolish the pseudovirus entry (*68, 121*) (**Fig. 9**). Recently, we have discovered a number of alpha and beta CoVs including HKU4/5 (*122*)(Ge *et al.*, Co-infection of alphacoronaviruses in one bat community, in China. unpublished results). The established approaches will enable us to analyze the receptor usage of these CoV S proteins and understand the host range and potential interspecies transmission ability of these novel CoVs and finally predict the potential spillover probability of these viruses to humans or other hosts at a molecular level.

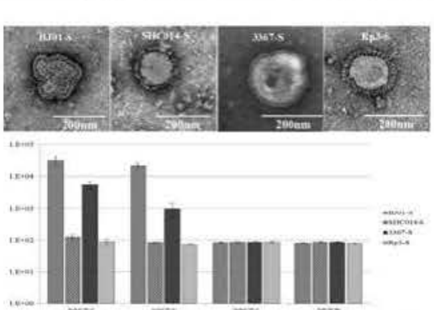


Figure 9: Top panel: HIV pseudovirus carrying spike proteins from human SARS-CoV (BJ01-S) and bat SARS-like CoV (SHC014-S, 3367-S and Rp-S). Bottom panel: Infectivity assay with the above pseudoviruses in HeLa cell lines expressing ACE2 from human, civet and bat.

<u>*In vitro*</u> *cell lines & Humanized mouse model:* We have developed primary cell lines and transformed cell lines from 9 bat species using kidney, spleen, heart, brain and intestine. We have used these for virus isolation, infection assays and receptor molecule gene cloning. We also have a large number of cell lines from humans and animals that we will use for virus infectivity assays. We have obtained a letter of support from Dr Ralph Baric, who is keen to collaborate with us initially to infect his humanized mouse model with our bat SL-CoV that uses ACE2, and subsequently to use other CoVs we identify (see **Dr Ralph Baric, Letter of Support**).

C3b) Receptor-mutant pseudovirus binding assays: We will amplify ACE2, DPP4 or other receptor genes of human and bats and clone them into eukaryotic expression vector pcDNA3.1 to construct cells expressing these molecules. We will amplify full length spike genes (S) of bat-CoVs detected from different bat species. The full length S gene, particularly RBDs, will be codon optimized, then cloned into eukaryotic expression vector pcDNA3.1 (*68, 123*). For packaging pseudovirus, S-expressing plasmids (or empty vector control) and pHIV-Luc (pNL4.3.Luc.R^- E^- -Luc) bone plasmid will be co-transfected into 4 x 10^6 293T cells using calcium phosphate transfection system (Promega), after 8 hours, replacing the medium with fresh medium, and supernatants will be harvested at 48 hours post transfection and separated from cell debris by centrifugation at 3,000g, then by passing through a 0.45μm filter (Millipore). The filtered supernatants will be stored at -80°C in aliquots until the use. We will use prepared pseudoviruses bearing different S proteins to infect human and bat ACE2 or DPP4 receptor expressing cells (in Hela cell model), 24 hours post infection, receptor usage by different S proteins will be determined by measuring luciferase activities. We will also induce site mutations in S proteins using site-directed mutation method, then do receptor-mutant pseudovirus binding assays. Pseudovirus infectivity on different human cell lines (A549, 293T, Caco, Huh7, and etc), primary and immortalized bat cell lines (listed below) and other mammalian cell line (mouse, pig, hamster, monkey, and ect) will be also determined by luciferase assay. <u>The results will provide information whether bat-CoVs could use known bat and human ACE2, DPP4 or other known CoV receptors to enter cells, and allow us to determine critical receptor binding sites, viral host range, and to better predict the capacity of our CoVs to infect people</u>.

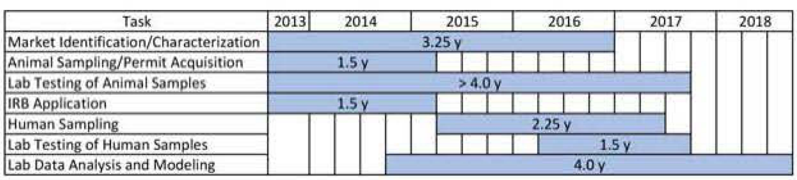
C3c) *In vitro* studies: We will isolate bat-CoVs using Vero E6 cell (susceptible SARS-CoV and MERS-CoV) and primary or transformed bat cell lines that we have developed from *Myotis davidii, Rhinolophus sinicus, Myotis chinensis, Rousettus leschenaultia* and other bats of China (*124, 125*). CoV PCR-positive bat samples (in 200 µl buffer) will be 3,000-12,000 rpm gradient centrifuged, and supernatant will be diluted at 1:10 in DMEM medium, then added to cells, incubated at 37°C for 1 h, the inoculum removed and replaced by fresh DMEM medium with 2% fetal calf serum, and cells checked daily for cytopathic effect (CPE). Double dose triple antibiotics (penicillin 200 IU/ml, streptomycin 0.2 mg/ml, amphotericin 0.5 µg/ml-Gibco) will be included in all culture media. Three blind passages will be carried out for each sample and the culture supernatant and cell pellet examined for presence of virus by RT-PCR using primers targeting the RdRp or S gene after each passage (*28, 126*). Live bat-CoVs will be sequenced to confirm viral receptor and by comparing viral infection in ACE2 or DPP4 expression cells and virus infectivity and replication on different human cell lines (A549, 293T, Caco, Huh7, and etc), bat cells and others (mouse, pig, hamster, monkey) using plaque assay, real time-PCR, and Immunological Fluorescence Assay (IFA). These *in vitro* assays will be used to test viral host species range and transmission possibility of bat-CoVs to human and other mammal, as predicted by our GLM and matrix models.

C3d) Humanized mouse *in vivo* infection experiments: To evaluate pathogenicity of bat-CoVs we will perform *in vivo* infection experiments in humanized mice modified to carry human ACE2 or DPP4 gene in the Wuhan Institute of Virology BSL-3 animal facility. We will passage isolated bat-CoVs in permissive cells twice, administer a specific inoculum (e.g. 1×10^6 TCID50) to intranasally or intraperitoneally. Mouse body temperature will be monitored with implanted microchips (LifeChip Bio-thermo, Destron Fearing), and mice will be weighed and observed for clinical signs of illness daily. Dead or moribund mice will be euthanized, organs harvested and sectioned. Live animals will be euthanized at three weeks post-inoculation and organs harvested. We will test for neutralizing antibodies against bat-CoVs on days 10, 15 and 21 pi. We will collect nasal washes, oral swabs, and rectal swabs, and urine every two days and quantify virus using qRT-PCR. We will conduct routine histology, immunohistochemistry, qRT-PCR, and virus isolation on tissues. This work will provide information about viral pathogenicity, tissue tropism, transmission route, and infection symptom.

C3e) Binding affinity assay: The recombinant S proteins and receptor molecules (e.g. ACE2 or DPP4) will be expressed in insect cells or eukaryotic cells. Octet RED platform (ForteBio, Menlo Park, CA)) will be used to perform binding affinity kinetics experiments. Streptavidin-coated sensor tips from Fortebio will be used to capture biotinylated S protein onto the surface of the sensor. After reaching baseline, sensors will be moved to the association step containing indicated concentrations of wild or mutant receptor molecules diluted with kinetics buffer for 30 min and then dissociated for 30 min at 25°C. Binding affinity will be determined by collecting the dissociation constants KD, Kon (association-rate), and Koff (dissociation-rate) determined by fitting binding chromatogram data with the Octet®User Software.

C3f) Potential pitfalls and solutions: Through our targeted sampling in China, we may only identify a small portion of the huge diversity of bat-CoVs in bat populations. To resolve this, we plan to expand our sampling locations to include samples from across SE Asia and improve our detection methods targeting more virus sequences. We will also synthesize the S genes based on the published data for viruses we do not obtain. Virus isolation may be a big challenge for this specific aim. In our previous work, we have isolated a number of novel bat viruses including adenovirus, reovirus and SARS-like CoV and have refined and optimized our methods for virus isolation. We will also attempt to construct additional bat cell lines which are lacking interferon response or over expressing the receptor molecules and more susceptible for virus infection to increase isolation success.

D. TIMELINE & MANAGEMENT PLAN:



This project will take 5 years to complete. The initial phase will involve filing the IRB application, identifying sampling sites, and conducting animal sampling and testing. Mid-project efforts will involve initial human sampling, analyses of lab results and production of models. The final phase will involve testing human and wildlife samples and analyses and modeling to maximize results. **Project Management:** Funds will be managed via subcontracts originating with EcoHealth Alliance, which is an A133 (low risk)-audited 501 (c) 3 organization specializing in international research on emerging diseases. PI Daszak will oversee all aspects of the project management. He is an experienced manager, with over 15 years of federally-funded research experience. Prof. Shi, based at the Wuhan Institute of Virology, will oversee all laboratory testing and analyses. Prof. Shuyi Zhang will manage field sampling work. EcoHealth Alliance staff will manage all modeling and analytical approaches (Aims 1 & 2). Communication will be via monthly video-conferences using EHA's NIH ARRA-funded video-conference facility. Travel budget has been requested to enable regular face-to-face meetings for all key staff.

PROTECTION OF HUMAN SUBJECTS:

HUMAN SUBJECTS RESEARCH

1. **Risk to subjects**

This project is a study of human exposure to animal coronaviruses in southern China. Subjects will be enrolled on a voluntary basis and a single interview and sample collection will be conducted. Informed consent will be obtained. People found to be infected with an animal coronavirus will be followed up after 6 months with a secondary interview and collection of biological specimens to determine whether infection is persistent and exposure is ongoing. Primary subjects will be male or female adults who are highly exposed to wildlife through hunting, butchering, or general handling in the context of live animal markets or restaurants that prepare and serve wild animals. The study population will be selected in Shanghai, Yunnan, Fujian, and Guangxi provinces, China, and will be open to people of all ethnicities that fit the subject criteria. We will target human subjects, comprising 220 subjects (market workers and hunters) and 400 controls from the general population in Yunnan, Fujian, and Guangxi provinces plus 600 subjects in Shanghai (total enrolled: 2460). The market types are defined in **Specific Aim 1, Human exposure to CoVs**. There are no data to suggest an ethnic bias for coronavirus exposure or infection, therefore subjects will be enrolled based on exposure criteria, though subjects will not be excluded based on ethnicity or gender. We will endeavor to have an equal number of men and women, if the composition of animal vendors in markets allows.

Sources of Materials: Samples to be collected and screened for coronaviruses include blood, saliva and stool samples. 10 mL of blood will be collected from each subject. Subjects will also be asked to provide saliva and stool in sterile containers. An initial sample collection and interview will be performed by trained medical personnel from the local CDC under the provincial Public Health Bureau. Sample collection will be done once in years 2-4 of the study. Samples will be screened for coronaviruses using PCR and an ELISA at the appropriate CDC microbiology lab or at the Wuhan Institute of Virology. Samples that test positive for coronavirus or antibodies to coronavirus will be followed up after 6 months with a secondary interview designed to determine the current level of exposure to wild animals, and whether exposure at the current level was consistent between the first and subsequent interview. Repeated clinical samples will also be collected and tested for coronaviruses. In all instances, volunteers will be given a medical exam and informed of their test results.

Potential risks: The potential risks to study participants resulting from study participation are minimal. The volume of blood being collected is within normal safety limits. The interview questions will be designed to assess exposure risk, and may ask personal questions, but surveys will be done in private and anonymized to protect privacy. There may be some stress to subjects who are informed that they have been exposed to an animal virus, but counseling will be available and options for medical care will be included in the discussion.

2. Adequacy of protection against risks

Recruitment and informed consent: Prospective study participants will be identified by the research team at each site in partnership with CDC personnel. The team will be thoroughly trained on communicating the research objectives and will be able to address any questions that potential subjects may have. Both written and oral descriptions of the study will be provided in Chinese (in Mandarin or via an interpreter in local dialect if necessary) as part of the informed consent process. Contact details of the collaborators at local CDCs and the study PI will be provided to all subjects, and CDC personnel on the research team will be available on site to answer questions from the study subjects. Test results will be communicated to each subject and counseling offered to minimize stress.

3. Potential benefits to Subjects and Others

There are potential benefits to the study subjects including receiving a physical exam/health check from a medical officer and the potential benefit of identifying an occupational health hazard. At the conclusion of the study, we will deliver an educational workshop for high risk individuals (open to study subjects and non-study subjects) describing the health benefits of using PPE and hand-washing during animal handling activities throughout the day.

4. The importance of knowledge to be gained. There are valuable potential benefits to the general public from the knowledge to be gained by this study, as it may identify sources of zoonotic coronaviruses in the market system or which are commonly hunted. Avoidance of these animals or extra care when handling them may substantially reduce the risk of CoV (and other zoonotic pathogen) transmission.

INCLUSION OF WOMEN AND MINORITIES:

Inclusion of Women: This proposal will enroll men and women as study subjects. Depending on local gender composition of animal vendors, we will make every effort to have men and women equally represented in this study.

Inclusion of Minorities: Subjects will be enrolled in this study without regard to ethnicity. Occupational exposure to wildlife in a market, hunting, or butchering context will be the primary criteria for identifying subjects.

Program Director/Principal Investigator (Last, First, Middle): Daszak, Peter

Targeted/Planned Enrollment Table

This report format should NOT be used for data collection from study participants.

Study Title: The ecology of bat coronaviruses and the risk of future coronavirus emergence.

Total Planned Enrollment: 2460**

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino	,0	0	0
Not Hispanic or Latino	1,230	1,230	2,460
Ethnic Category: Total of All Subjects *	1,230	1,230	2,460
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	1,230	1,230	2,460
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	0	0	0
White	0	0	0
Racial Categories: Total of All Subjects *	1,230	1,230	2,460

* The “Ethnic Category: Total of All Subjects” must be equal to the “Racial Categories: Total of All Subjects.”

(** all study subjects will be enrolled at foreign sites in China)

INCLUSION OF CHILDREN:

Inclusion of Children: Children will not be included in this study. Children do not normally work in wildlife markets, and are not normally involved in the wildlife trade in China.

VERTEBRATE ANIMALS:

1. Detailed description of animal use.
All work with vertebrate animals will be conducted in China.
Capture and sampling techniques for all wild animals described in this study have been previously approved by UC Davis IACUC (Mazet and Epstein; UC Davis 15898; current).

Experimental work using humanized mice will be conducted at the Center for Animal Experiment Biosafety 3 lab of Wuhan University at the School of Medicine in Wuhan, China. The Center is AAALAC accredited and has both an Institutional Biosafety Committee and an Institutional Animal Care and Use Committee. Animals will be housed in a BSL-3 facility and will be under the care of a full-time veterinarian. We will submit our protocols for IACUC approval should this proposal be funded. Conditions for animal use are described below.

Note: The majority of wild animals captured and sampled will be done using non-destructive, techniques. In a small number of instances (~ 2 bats per species), where intestine and lung tissue is required to establish cell lines, animals will be humanely euthanized and a necropsy performed according to accepted protocols (see euthanasia section)

Bat capture. Free-ranging bats will be captured using either a mist net or harp trap. The net system is manned by two people during the entire capture period, and bats are removed from the net as soon as they become entangled to minimize stress and prevent injury. In the Co-PI's (Dr. Epstein) experience, a maximum of 20-30 bats can be safely held and processed by a team of three people per trapping period. Duration of trapping will depend on the capture rate. Bats are placed into a pillowcase or small cloth bag and hung from a branch or post until samples are collected. Bats are held for a maximum of six hours.

Wild rodent capture. Free-ranging rodents will be captured through pit traps and box traps; captive rodents, including resident free-ranging wild rats/rodents in markets, will be manually captured or captured through traps. Traps will be checked a minimum of once daily in the morning. If adverse weather (extreme heat, rain) is expected or researchers are working in areas where predation is common, traps will be checked more frequently, and closed during the adverse weather. Handling of rodents will involve morphometric measurements. Captive and wild rodent sampling procedures (including anesthesia if necessary), will involve manual restraint, venipuncture, mucosal swabs, fecal, urine, and external parasite collection. Following capture, small animals will be restrained with a fine mesh bag to minimize entanglement, taking precautions to ensure the animals are not traumatized by the hoop of the net or through net removal. Larger rodents will be restrained for sampling in specialized squeeze-cages, allowing adjustments appropriate to the size of the animal. Squeeze-cages consist of a wooden frame with a plasticized wire bottom and a Plexiglas shield used to press the animal, while ensuring visible communication between the field veterinarian and the animal. Once squeezed, a rod is inserted to keep the plastic shield in place. The box is then inverted, allowing sampling to be conducted through the open wire bottom and abdomen of the animal when the animal is safely immobilized. Anesthesia for small rodents will be conducted using plastic tubes, with the animals transferred directly from the traps to the tubes containing a cotton swab soaked in ether, isoflurane, or methoxyflurane for anesthetic induction. For larger rodents, chemical restraint and anesthesia (ketamine alone, or ketamine combined with xylazine) will be applied either through the squeeze cages by syringe if applicable.

Laboratory mice. Lab mice will be sourced commercially by the Wuhan Center for Animal Experiment at Wuhan University.

Sample Collection. Bats will be manually restrained during sampling.
Bats: Depending on the species and size of bat, swabs will be taken from the oropharynx, urogenital tract, and rectum. Fresh feces will be collected if available, in which case a rectal swab will not be collected. Blood will be collected from fruit bats either from the cephalic vein or from the radial artery or vein using a 25 gauge needle and 1cc syringe. Blood will be collected from bats weighing less than 100g according to published techniques (*126*).

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Rodents: Rodents will be anesthetized prior to sampling.
Once anesthetized a small blood sample will be collected using a capillary tube placed into the retro-orbital sinus. Only trained technicians will perform retro-orbital bleeding and it will only be performed on anesthetized rodents. Femoral or jugular venipuncture may be used for larger rodents (e.g. rats). In all rodents, blood volumes of no more than 1% of body weight will be withdrawn. (example 0.2 ml blood from a 20 gram rodent).

Civets and other small mammals: Anesthesia will be used to restrain small free ranging mammals according to published protocols. Animals will be monitored continuously while recovering from anesthesia. Animals that are sampled in the marketplace, and that may potentially be consumed, will not be anesthetized. Manual restraint will be used and blood will be drawn from the femoral artery or saphenous vein.

Laboratory Mice. Humanized mice will be bred at the University of Wuhan. Mice will be inoculated with a specific dose (e.g. $1x10^6$ TCID50) of virus through different routes (intranasally and intraperitoneally). Mouse body temperature will be monitored with implanted temperature sensing microchips (LifeChip Bio-thermo, Destron Fearing), and mice will be weighed daily. Animals will be observed daily for clinical signs of illness. Moribund mice will be euthanized, according to AVMA recommendations. Live animals will be euthanized at three weeks post-inoculation and organs harvested. We will collect sera on days 10, 15 and 21 to test for neutralizing antibodies against bat CoVs. We will collect nasal washes, oral swabs, and rectal swabs, and urine every two days. These are minimally invasive procedures, and will be performed by experienced lab technicians under the supervision of a full-time veterinarian.

2. Justify use of animals, choice of species, numbers to be used. Species and number used in study: The purpose of this study is to conduct multi-regional surveillance in large populations of animals to detect coronaviruses that may pose a risk to the health of both humans and animals. The experimental work is designed to understand the ability of bat coronaviruses to bind to human receptors. Because we don't have prevalence estimates for novel strains of coronaviruses, we assume a conservative estimate of 10% prevalence. SARS-like coronaviruses have been found in between 10% and 38% of bats studied (*4, 25*). A 10% in wild populations of bats would require a sample of 30 individuals per species in order to ensure detection of an infected individual with 95% confidence. **Wild bats**: We will sample 30 individuals from 30 different species in each province in China (2 per species euthanized for organ tissue); representing but not limited to the following families: *Rhinolophidae, Hipposideridae, Vespertillionidae, Mollossidae,* and *Pteropodidae*, all of which are present in Southern China and potentially in the wildlife markets. **Bats in wet markets:** We will opportunistically sample a wide variety of insectivorous and frugivorous bats according to what is present in markets. In addition to bats, we will sample civets, raccoon dogs, rats, bandicoots, bamboo rats, and other rodents present in the markets that may act as intermediate hosts. Numbers of animals sampled from markets will be limited to animal availability. In every situation, sampling of wildlife will be conducted in the most humane manner while minimizing the impacts on individual animals and their wild populations. In cases where feces are collected for testing, non-invasive techniques will be used. In all instances, the fewest number of animals will be sampled that will provide valid information and statistical inference for the pathogen and disease of interest and every effort will be made to minimize stress and discomfort for the animal.

A small number of bats (maximum 2 per species) representing each of the species in this study may be euthanized in order to collect lung and intestinal tissue required for characterizing coronavirus receptors. Voucher specimens may also be collected at the discretion of the team leader for the accurate identification of species using molecular methodology.

Humanized mice for experimental infection for Specific Aim 3: In order to understand whether bat coronaviruses that utilize receptors found in people have the potential to infect people, we will use Swiss albino mice (standard breed at Wuhan University) that have been genetically modified to have human receptors. We'll infect them with cultured bat coronaviruses and determine which organs become infected and whether these mice are capable of shedding infectious virus. Humanized mice will be genetically modified to carry human ACE2 or DPP4 gene will be used to evaluate pathogenesis of CoVs. We cannot anticipate exactly how many viruses we will find that are candidates for experimental models, however we estimate that we will use
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four adult mice (2 male, 2 female) per virus and that we will identify approximately 20 viruses that will be used for mouse infection experiments. This will require a total of 80 mice over the study period.

3. Provide information on veterinary care. For wild caught animals, there is no specific veterinary care that is appropriate, nor will clinical veterinary facilities be available. Animals that are injured during the capture or sampling process will be assessed by an experienced team leader, and if the animal is determined to be unlikely to survive if released, it shall be euthanized humanely (see euthanasia section). Animals will be released within hours of capture. In the markets, animals will be sampled using manual restraint or anesthesia. Animals will be returned to vendors after sampling, or, if wild caught in the markets (e.g. rodents), they will be released in the area outside the marketplace.

Laboratory mice will be housed in the BSL-3 small animal facility Center for Animal Experiment at Wuhan University. Experimental animals will be regularly monitored by experienced staff and a supervising veterinarian. The animal facility operates 24 hours a day and has full-time veterinarians on staff. All animals will be provided with food and water ad libitum and will otherwise receive standard care.

4. Procedures for ensuring animal comfort, lack of distress, pain, or injury: Animals will not be held longer than 6 hours. Co-PIs, Drs. Epstein and Olival have extensive experience in capture, anesthesia, and sampling wildlife, including bats. In our experience, bats and rodents tolerate the described procedure well. Mist nets will be attended continuously during capture periods, and bats will be extracted from the net as soon as they become entangled. This will minimize stress and injury from entanglement. Bats will be placed individually in cotton bags and hung from tree branches while awaiting processing and during recovery. The bags are sufficiently porous as to allow for ventilation and are designed for bat capture. The enclosed environment seems to calm the bats, as they do not struggle once inside, but they hang quietly. Animals will be monitored by a veterinarian or experienced field team member during all stages of capture, processing, and release. Animals will be kept in a cool place while in the pillowcases. Rodent traps will be set overnight and all traps will be checked in the morning while it still cool outside. Rodents will be kept in a cool, shaded environment during sampling and will be released within 10 hours of capture.

The procedures used in this experiment (blood draw, nasal, oral, and rectal swabs) are minimally invasive, however, mice that show signs of morbidity post-infection will be examined and euthanized according to AVMA standards (see below).

5. Euthanasia: In the event of injury to an animal that results in pain and suffering, and reasonable veterinary care is unavailable, the animal will be euthanized by a veterinarian or trained field team member using ketamine injected intramuscularly 37.5mg/kg and sodium pentobarbital injected intravenously at a dose of 1.0ml per 5kg injected intravenously. This protocol is in accordance with the AVMA euthanasia report (2007). Any animal that is euthanized using a chemical agent will be disposed such that it will not be permitted to enter the food supply either through markets or hunting.

SELECT AGENT RESEARCH/BIOHAZARDS. No select agent research as of 5/25/12

SARS-CoV caused outbreaks with significant case fatality rates, and there are no vaccines available for this agent. SARS-CoV is classified as a BSL-3 agent. The work proposed in this application will involve two aspects: field work and laboratory work. Fieldwork involves the highest risk of exposure to SARS or other CoVs, while working in caves with high bat density overhead and the potential for fecal dust to be inhaled. There is also some risk of exposure to pathogens or physical injury while handling bats, civets, rodents or other animals, their blood samples or their excreta. The Co-PI is a veterinarian with extensive experience working with wildlife species and high-biosecurity pathogens (Nipah virus, ebolavirus, SARS), and great care will be taken in the field to limit the risk of accidental exposure to known or unknown animal pathogens. We have strict procedures for handling bats and working with samples from them as they are secured in the field and transported to the lab. Field team members handling animals will be trained to utilize personal protective equipment and practice proper environmental disinfection techniques. This includes wearing coveralls or dedicated clothing, nitrile gloves, eye protection, and a P95 or P100 respirator. All field clothing and equipment will be disinfected using Virkon disinfectant. All biological waste from field surveys will be disposed of in the appropriate container (sharps box or an autoclave bag) and will be autoclaved at local hospitals or university labs. All personnel will be vaccinated against rabies and have a neutralizing antibody titer, in accordance with WHO and CDC recommendations. Field teams will carry rabies boosters in the field and will receive a booster in the event of a potential rabies exposure.

Field safety protocol: Our procedures to deal with bites, needle-sticks etc. are as follows: The wound is washed thoroughly with soap and water to clean away dirt and debris, then vigorously scrubbed with a sterile gauze bandage and benzalkonium chloride for 5 minutes. If bleeding, pressure is applied with a sterile bandage for until bleeding has stopped. If the wound continues to bleed, medical attention at the nearest hospital is sought. The bat from which the bite or exposure originated is identified, and the samples collected from it labeled on the data sheet that these were involved in an exposure. Our procedures require that the person potentially exposed reports to a major hospital within 24 hours to have wound examined and receive a rabies booster (as per WHO/CDC protocols). The laboratory work is lower risk, as samples placed in lysis buffer will be non-infectious. Samples placed in viral transport medium and frozen will be stored at ultra-low temperatures (-86C) until viral isolation is required. Serum will be heat inactivated (56C for 30 min) prior to testing.

Lab biosafety: Wuhan Institute of Virology and the Wuhan University Center for Animal Experiment BSL-3 lab have an Internal Biosafety Committee and are accredited BSL-2 and BSL 3 laboratories. All experimental work using infectious material will be conducted under appropriate biosafety standards. Disposal of hazardous materials will be conducted according to the institutional biosafety regulations.

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CONSORTIUM/CONTRACTUAL ARRANGEMENTS:

Consortium/Contractual Arrangements

This project is a multi-institutional collaboration led by EcoHealth Alliance, New York (Daszak, PI), which will subcontract funds to two institutions: the East China Normal Univerisity (Dr S. Zhang) and the Wuhan Institute of Virology (Dr. Z. Shi), which are both foreign institutions. Dr. Daszak has over 15 years previous experience managing collaborative projects including two R01s on Nipah virus ecology that involved 5 separate foreign institutions, a 5-year NSF/NIH Ecology of Infectious Disease award on West Nile virus which involved multiple subcontractees, an R01 on bat viral discovery that involves multiple international contracts, and a multi-million dollar p.a. contract from USAID that involves 12 international partners. The applicant organization (EcoHealth Alliance) is justified in taking the lead on this project because it specializes in understanding the ecological, and virological processes underlying zoonotic disease emergence. Dr Daszak has conducted significant preliminary work on this issue including 10-years of research on the ecological and related factors of the emergence of SARS and 11-years of work in China. The subcontractees will work on specific issues and areas in which they have proven expertise. These areas are: human and animal field sampling (East China Normal University, Dr. Zhang) and viral discovery, pathogenesis as well as sample storage and shipping (Wuhan Institute of Virology, Dr. Shi). Dr Daszak has launched and co-directed a joint institute in China with Dr Zhang, and has been involved in contractual arrangements with ECNU for 8 years. Drs Shi, Zhang, and Daszak have collaborated together since 2002 and have been involved in running joint conferences, and shipping samples into and out of China.

上海市疾病预防控制中心
Shanghai Municipal Center for Disease Control and Prevention
地址：中国上海市中山西路 1380 号, 200336 Add:1380 Zhongshan Rd.(w) Shanghai, 200336 P.R.China
电话(Tel): +86-21-62758710 传真(Fax): +86-21-62756323 网址(Website): http://www.scdc.sh.cn

Dr. Peter Daszak
President
EcoHealth Alliance
460 W 34th St. 17th Floor
New York, NY 10001
USA

Dear Dr. Daszak,

I am writing in response to a request for collaboration on an upcoming NIAID funded R01 entitled "Understanding the risk of bat coronavirus emergence." The Shanghai CDC has a high interest in working with EcoHealth Alliance and its scientists in identifying and preventing the transmission of bat coronaviruses to human populations.

The Shanghai CDC recognizes the mutual benefits to be gained through research cooperation and a successful partnership with EcoHealth Alliance in the field of identification and prevention of zoonotic disease transmission. It is vital to not only identify the diseases themselves, but also identify high-risk human populations and the actions that put them at risk for infection along with evaluating approaches to intervention and disease management.

Understanding and preventing exposure and transmission of zoonotic diseases from wildlife to humans remains a high priority for prevention of pandemics. In our discussion with EcoHealth Alliance, we have agreed to participate in activities that will strengthen the ability of China and other countries in the region to respond to the outbreak of epidemic diseases, particularly those of animal origin. To assist in this study, we will provide participating laboratories in China with human epidemiological information, both new and archived, to support research in bat coronaviruses.

We at the Shanghai CDC look forward to our collaboration with the EcoHealth Alliance team and working further on this worthwhile study.

Sincerely

吴凡

Fan Wu, M.D.
Director General
Shanghai Municipal Center for Disease Control and Prevention

WUHAN INSTITUTE OF VIROLOGY
The CHINESE ACADEMY OF SCIENCES

Address: Xiaohongshan 44, Wuchang, Wuhan 430071, Hubei, P. R. China
Tel: +86-27-87198117 Fax: +86-27-87198072 http://www.whiov.ac.cn

May 23, 2013

To whom it may concern:

On behalf our Institute, I am very pleased to express my strong support for Dr. Zhengli Shi for applying for the R01 entitled "Understanding the Risk of Bat Coronavirus Emergence" under the project managed by Peter Daszak, president of EcoHealth Alliance. Dr. Shi has extensive expertise in viral pathogen discovery. Since 2004, Dr. Shi's laboratory has discovered a variety of genetically diverse bat viruses including bat SARS-like coronavirus, bat adenovirus, and adeno-associated viruses. She has established a worldwide collaborative-group of leading experts on viral pathogens and ecology covering identification of emerging viruses, epidemiology on bat-borne viruses including Hendra and Nipah virus and SARS-coronavirus. Her work with Dr. Peter Daszak led to the discovery of bat SARS-like coronavirus in 2005.

Our Institute would provide all necessary support to Dr. Shi for accomplish the project if it is approved.

Sincerely yours,

Dr. Xinwen Chen
Director, Wuhan Institute of Virology
Chinese Academy of Sciences
Xiao Hong Shan, No. 44
Wuhan 430071 China
[redacted] (b) (6)

UNC SCHOOL OF PUBLIC HEALTH
DEPARTMENT OF EPIDEMIOLOGY

5/31/2013

Dr. Peter Daszak
President
EcoHealth Alliance
460 W 34th St. 17th Floor
New York, NY 10001
USA

Dear Dr. Daszak,

I am writing in response to a request for collaboration on an upcoming NIAID R01 grant entitled "Understanding the risk of bat coronavirus emergence." I agree that studies are definitely needed to identify the key risk factors and develop strategies that prevent the transmission of bat coronaviruses to human populations. Understanding and preventing exposure and transmission of zoonotic diseases from wildlife to humans remains a high priority for prevention of pandemics.

Our laboratory has developed a variety of animal models for understanding human coronavirus pathogenesis in vivo. We have developed transgenic mouse models in the C57BL/6 mice, expressing hACE2 in ciliated cells from the FOXJ1 promoter. Unlike other epithelial cell promoters (e.g., K18, hACE2 expression from FOXJ1 should be specific to the airway epithelium. FOXJ1 (hepatocyte nuclear factor-3/forkhead homologue 4; HFH-4) is a member of the forkhead/winged helix family of transcription factors whose expression is tightly restricted to cells possessing motile cilia or flagella. Inoculation of these mice with wild type SARS-CoV resulted in lethal respiratory tract infections characterized by high virus titers (>10^8 PFU/day 4), hemorrhage, severe pneumonia and acute respiratory distress syndrome between days 2-7 post infection (Fig 1). We also have aged and immunosenescent models that are highly vulnerable to synthetically reconstructed strains of SARS-CoV from early in the epidemic. This letter states my willingness to collaborate with your group to evaluate the in vivo pathogenesis of interesting bat and animal SARS-like coronaviruses.

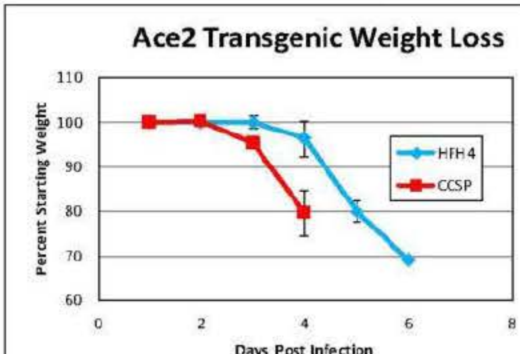


Figure 1. FOXJ1 ACE2 Transgenic Mice are Highly Susceptible to wildtype SARS-CoV Infection. Two different lines were infected with wildtype SARS-virus and clinical disease evaluated at different times postinfection.

It was a pleasure talking with you the other day. I believe your proposal asks fundamentally important questions in the evolution of new human coronaviruses from bats, contributes

dramatically to our understanding of coronavirus variation in natural populations, and provides key insights into the ecology of new emerging infectious diseases. Let me know if I can be of any additional assistance.

Sincerely,

Ralph S. Baric, Professor
Department of Epidemiology
Department of Microbiology and Immunology
Ph: (b) (6)
Email: (b) (6)

云南省地方病防治所

YUNNAN INSTITUTE OF ENDEMIC DISEASES CONTROL AND PREVENTION (YIEDC)

Dr. Peter Daszak
President
EcoHealth Alliance
460 W 34th St. 17th Floor
New York, NY 10001
USA

Dear Dr. Daszak,

I am writing in response to a request for collaboration on an upcoming NIAID funded R01 entitled " Understanding the risk of bat coronavirus emergence." The Yunnan Institute of Endemic Diseases Control and Prevention (EDC) has a high interest in working with EcoHealth Alliance and its scientists in identifying and preventing the transmission of bat coronaviruses to human populations.

The Yunnan EDC recognizes the mutual benefits to be gained through research cooperation and a successful partnership with EcoHealth Alliance, and long term colleague ZhengLi Shi, in the field of identification and prevention of zoonotic disease transmission. It is vital to not only identify the diseases themselves, but also identify high-risk human populations and the actions that put them at risk for infection along with evaluating approaches to intervention and disease management.

Understanding and preventing exposure and transmission of zoonotic diseases from wildlife to humans remains a high priority for prevention of pandemics. In our discussion with EcoHealth Alliance, we have agreed to participate in activities that will strengthen the ability of China and other countries in the region to respond to the outbreak of epidemic diseases, particularly those of animal origin. To assist in this study, we will provide participating laboratories in China with human samples, both new and archived, and support research in bat coronaviruses.

We at the Yunnan EDC look forward to our collaboration with the EcoHealth Alliance team and working further on this worthwhile study.

Sincerely,

Zhang Yunzhi
Yunnan Institute of Endemic Diseases Control and Prevention
Tel: [redacted] (b) (6) E-mail: [redacted] (b) (6)

地址：中国.云南.大理市文化路33号 电话：(Tel)：0872-2125196 传真：(Fax)：0872-2125437
Add: 33wenhua Rd., Dali City; Yunnan. P.R.China 邮编：(P O Box)：671000

第 页

广东省疾病预防控制中心

Guangdong Provincial Center for Disease Control and Prevention

Dr. Peter Daszak
President
EcoHealth Alliance
460 W 34th St. 17th Floor
New York, NY 10001
USA

Dear Dr. Daszak,

I am writing in response to a request for collaboration on an upcoming NIAID funded R01 entitled " Understanding the risk of bat coronavirus emergence." The Guangdong CDC has a high interest in working with EcoHealth Alliance and its scientists in identifying and preventing the transmission of bat coronaviruses to human populations.

The Guangdong CDC recognizes the mutual benefits to be gained through research cooperation and a successful partnership with EcoHealth Alliance in the field of identification and prevention of zoonotic disease transmission. This partnership will continue a successful five year relationship between the Guangdong CDC and EcoHealth Alliance. It is vital to not only identify the diseases themselves, but also identify high-risk human populations and the actions that put them at risk for infection along with evaluating approaches to intervention and disease management.

Understanding and preventing exposure and transmission of zoonotic diseases from wildlife to humans remains a high priority for prevention of pandemics. In our discussion with EcoHealth Alliance, we have agreed to participate in activities that will strengthen the ability of China and other countries in the region to respond to the outbreak of epidemic diseases, particularly those of animal origin. To assist in this study, we will provide participating laboratories in China with human samples, both new and archived, and support research in bat coronaviruses.

We at the Guangdong CDC look forward to our collaboration with the EcoHealth Alliance team and working further on this worthwhile study.

Sincerely,

Ke Changwen

Ke Changwen

地址：广州市番禺区大石街群贤路160号　　邮编：511430
Add: Qunxian Road, Dashi Town, Panyu District, Guangzhou, Guangdong, China, 511430
电话：（Tel）：020-31051000　　传真：（Fax）：020-31051502
电子邮箱（E-mail）：webmaster@cdcp.org.cn　　网址（Website）：http://www.cdcp.org.cn

华東師範大學

科学与技术跨学科高等研究院

Institutes for Advanced Interdisciplinary Research , ECNU

25 May 2013

Dr. Peter Daszak
President
EcoHealth Alliance
460 W 34th St. 17th Floor
New York, NY 10001
USA

Dear Dr. Daszak,

As Dean of Institutes for Advanced Interdisciplinary Research, I am delighted at the prospect of our continued collaboration on the NIAID funded R01 "Understanding the Risk of Bat Coronavirus Emergence."

Since 2005, our organizations have collaborated via our School of Life Science. We have a joint-MOU as well. I have enjoyed our close working relationship with EcoHealth Alliance especially on issues related to emerging infectious diseases and health.

Our collaborations include past and current research projects in Guangzhou, Guangxi, Yunnan, Hainan, and Shanghai as well as capacity building, training, and over 20 joint publications including Science papers, which have led to increased understanding of the ecology of disease dynamics and garnered invaluable data towards predicting and preventing zoonotic disease emergence. My field and laboratory teams based in Beijing, Shanghai, Guangxi, and Guangzhou are ideally positioned to conduct both research and surveillance as we work towards reducing the risk of zoonosis in China.

In our discussion with EcoHealth Alliance, I have agreed to participate in activities that will strengthen the ability of China and other countries in the region to respond to the outbreak of epidemic diseases - particularly those of animal origin.

I look forward to our continued collaboration and the results of this exciting and timely project.

Sincerely,

Zhang Shu-Yi

Dr. Zhang Shu-Yi
Dean of Institutes for Advanced Interdisciplinary Research
East China Normal University
B319, Science Building, 3663, North Zhongshan Road,
Shanghai 200062 China
[redacted] (b) (6)

RESOURCE SHARING PLAN:

Data Sharing Plan*:* Sequence data will be made publicly available via GenBank, and shared when requested by other scientists, as soon as a publication is in press. Viral isolates will remain at the Wuhan Institute of Virology initially. Isolates, reagents and any other products, should they be developed, will be made available to other NIH-funded researchers via applicable Wuhan Institute of Virology and EcoHealth Alliance Material Transfer Agreements and/or licensing agreements.

Sharing Model Organisms*:* We do not anticipate the development of any model organisms from this study. Should any be developed, they will be made available to other NIH-funded researchers via applicable Wuhan Institute of Virology and EcoHealth Alliance Material Transfer Agreements and/or licensing agreements.
Genome Wide Association Studies (GWAS): N/A

PHS 398 Checklist

OMB Number: 0925-0001

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

[X] New [ ] Resubmission [ ] Renewal [ ] Continuation [ ] Revision

Federal Identifier: GRANT11418218

2. Change of Investigator / Change of Institution Questions

[ ] Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

[ ] Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes [ ] No [ ]

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes [ ] No [ ]

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

5. * Disclosure Permission Statement

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

☒ Yes ☐ No

RESEARCH **Federal Award Date:** 06/10/2015

Department of Health and Human Services
National Institutes of Health
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI110964-02
FAIN: R01AI110964

Principal Investigator(s):
PETER DASZAK, PHD

Project Title: Understanding the Risk of Bat Coronavirus Emergence

Aleksei Chmura
President
460 West 34th Street
17th Floor
New York, NY 100012317

Award e-mailed to: [redacted] (b) (6)

Period Of Performance:
Budget Period: 06/01/2015 – 05/31/2016
Project Period: 06/01/2014 – 05/31/2019

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $630,445 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to ECOHEALTH ALLIANCE, INC. in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI110964. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Laura A. Pone
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI110964-02

Award Calculation (U.S. Dollars)

Federal Direct Costs	$502,293
Federal F&A Costs	$128,152
Approved Budget	$630,445
Total Amount of Federal Funds Obligated (Federal Share)	$630,445
TOTAL FEDERAL AWARD AMOUNT	$630,445
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$630,445

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
2	$630,445	$630,445
3	$611,090	$611,090
4	$597,112	$597,112
5	$581,646	$581,646

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name: Allergy, Immunology and Transplantation Research
CFDA Number: 93.855
EIN: 1311726494A1
Document Number: RAI110964A
PMS Account Type: P (Subaccount)
Fiscal Year: 2015

IC	CAN	2015	2016	2017	2018
AI	8472350	$630,445	$611,090	$597,112	$581,646

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M51C / **OC**: 414E / **Released**: [redacted] (b) (6)L 06/09/2015
Award Processed: 03/23/2015 01:36:12 PM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI110964-02

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI110964-02

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain

references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI110964. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI110964-02

This Notice of Award (NoA) includes funds for consortium activity with **Wuhan Institute of Virology - CHINA** awarded in the Total Costs amount of **$139,015** (**$128,718** Direct Costs + **$10,297** F&A Costs).

Future year commitments are as follows:

Year 3 Total Costs: $159,122
Year 4 Total Costs: $159,122
Year 5 Total Costs: $159,122

This Notice of Award (NoA) includes funds for consortium activity with **East China Normal University - CHINA** awarded in the Total Costs amount of **$72,684** (**$67,300** Direct Costs + **$5,384** F&A Costs).

Future year commitments are as follows:

Year 3 Total Costs: $54,117
Year 4 Total Costs: $42,300
Year 5 Total Costs: $32,454

Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants.nih.gov/grants/policy/nihgps_2013/nihgps_ch15.htm#_Toc271265264.

The written agreement with the consortium must address the negotiated arrangements for meeting the scientific, administrative, financial and reporting requirements for this grant.

No foreign performance site may be added to this project without prior approval of the National Institute of Allergy and Infectious Diseases.

Although a specific amount has been awarded for each consortium, the grantee retains standard rebudgeting authorities.
~~~~~~~~~
Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

o A list of the new and/or additional Agent(s) that will be studied;
o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.
~~~~~~~~~

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Laura A. Pone
Email: [redacted] (b) (6) **Phone**: [redacted] (b) (6) **Fax**: 301-493-0597

Program Official: Erik J. Stemmy
Email: [redacted] (b) (6) **Phone**: [redacted] (b) (6)

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI110964-02

INSTITUTION: ECOHEALTH ALLIANCE, INC.

Facilities and Administrative Costs	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	44.1%	44.1%	44.1%	44.1%
F&A Cost Base 1	$290,594	$276,094	$274,594	$270,694
F&A Costs 1	$128,152	$121,757	$121,096	$119,376

A. COVER PAGE

Project Title: Understanding the Risk of Bat Coronavirus Emergence	
Grant Number: 5R01AI110964-02	**Project/Grant Period:** 06/01/2014 - 05/31/2019
Reporting Period: 06/01/2014 - 05/31/2015	**Requested Budget Period:** 06/01/2015 - 05/31/2016
Report Term Frequency: Annual	**Date Submitted:** 05/01/2015
Program Director/Principal Investigator Information: PETER DASZAK , PHD BS **Phone number:** [redacted] (b) (6) **Email:** [redacted] (b) (6)	**Recipient Organization:** ECOHEALTH ALLIANCE, INC. ECOHEALTH ALLIANCE, INC. 460 W 34TH ST 17TH FLOOR NEW YORK, NY 100012320 **DUNS:** 077090066 **EIN:** 1311726494A1 **RECIPIENT ID:** 07-049-7012
Change of Contact PD/PI: No	
Administrative Official: ALEKSEI CHMURA 460 W 34th St., 17th Floor New York, NY 10001 **Phone number:** [redacted] (b) (6) **Email:** [redacted] (b) (6)	**Signing Official:** ALEKSEI CHMURA 460 W 34th St., 17th Floor New York, NY 10001 **Phone number:** [redacted] (b) (6) **Email:** [redacted] (b) (6)
Human Subjects: Yes HS Exempt: No Exemption Number: Phase III Clinical Trial:	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Zoonotic coronaviruses are a significant threat to global health, as demonstrated with the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, and the recent emergence Middle East Respiratory Syndrome (MERS-CoV). The wildlife reservoirs of SARS-CoV were identified by our group as bat species, and since then hundreds of novel bat-CoVs have been discovered (including >260 by our group). These, and other wildlife species, are hunted, traded, butchered and consumed across Asia, creating a largescale human-wildlife interface, and high risk of future emergence of novel CoVs.
To understand the risk of zoonotic CoV emergence, we propose to examine 1) the transmission dynamics of bat-CoVs across the human-wildlife interface, and 2) how this process is affected by CoV evolutionary potential, and how it might force CoV evolution. We will assess the nature and frequency of contact among animals and people in two critical human-animal interfaces: live animal markets in China and people who are highly exposed to bats in rural China. In the markets we hypothesize that viral emergence may be accelerated by heightened mixing of host species leading to viral evolution, and high potential for contact with humans. In this study, we propose three specific aims and will screen free ranging and captive bats in China for known and novel coronaviruses; screen people who have high occupational exposure to bats and other wildlife; and examine the genetics and receptor binding properties of novel bat-CoVs we have already identified and those we will discover. We will then use ecological and evolutionary analyses and predictive mathematical models to examine the risk of future bat-CoV spillover to humans. This work will follow 3 specific aims:

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces. We will examine if: 1) wildlife markets in China provide enhanced capacity for bat-CoVs to infect other hosts, either via evolutionary adaptation or recombination; 2) the import of animals from throughout Southeast Asia introduces a higher genetic diversity of mammalian CoVs in market systems compared to within intact ecosystems of China and Southeast Asia; We will interview people about the nature and frequency of contact with bats and other wildlife; collect blood samples from people highly exposed to wildlife; and collect a full range of clinical samples from bats and other mammals in the wild and in wetmarkets; and screen these for CoVs using serological and molecular assays.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk. We propose two competing hypotheses: 1) CoV host-range in bats and other mammals is limited by the
phylogenetic relatedness of bats and evolutionary conservation of CoV receptors; 2) CoV host-range is limited by geographic and ecological opportunity for contact between species so that the wildlife trade disrupts the 'natural' co-phylogeny, facilitates spillover and promotes viral evolution. We will develop CoV phylogenies from sequence data collected previously by our group, and in the proposed study, as well as from Genbank. We will examine co-evolutionary congruence of bat-CoVs and their hosts using both functional (receptor) and neutral genes. We will predict host-range in unsampled species using a generalizable model of host and viral ecological and phylogenetic traits to explain patterns of viral sharing between species. We will test for positive selection in market vs. wild-sampled viruses, and use data to parameterize mathematical models that predict CoV evolutionary and transmission dynamics. We will then examine scenarios of how CoVs with different transmissibility would likely emerge in wildlife markets.

Specific Aim 3: Testing predictions of CoV inter-species transmission. We will test our models of host range (i.e. emergence potential) experimentally using reverse genetics, pseudovirus and receptor binding assays, and virus infection experiments in cell culture and humanized mice. With bat-CoVs that we've isolated or sequenced, and using live virus or pseudovirus infection in cells of different origin or expressing different receptor molecules, we will assess potential for each isolated virus and those with receptor binding site sequence, to spill over. We will do this by sequencing the spike (or other receptor binding/fusion) protein genes from all our bat-CoVs, creating mutants to identify how significantly each would need to evolve to use ACE2, CD26/DPP4 (MERS-CoV receptor) or other potential CoV receptors. We will then use receptor-mutant pseudovirus binding assays, in vitro studies in bat, primate, human and other species' cell lines, and with humanized mice where particularly interesting viruses are identified phylogenetically, or isolated. These tests will provide public health-relevant data, and also iteratively improve our predictive model to better target bat species and CoVs during our field studies to obtain bat-CoV strains of the greatest interest for understanding the mechanisms of cross-species transmission.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: Professional Development.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

1) Conference and University lectures
• PI Daszak, and Co-investigators Olival and Shi gave >10 invited University lectures that included specific discussion of the current project and results.

2) Agency and other USG briefings
• NRC, 2015: Invited speaker, IOM Forum on public health preparedness, Interagency meeting on Medical Countermeasures. PI Daszak specifically reported on the findings from Year 1 of this project and the risk of SARS-like viruses causing future pandemics
• World Health Summit, Berlin 2014: PI Daszak was an invited panelist at a session on pandemic risk, and specifically reported the results and aims of this project
• International bat virus conference, Colorado, 2014: PI Daszak and Co-investigator Olival presented results from this study
• National Academies, Division of Earth & Life Studies, Spring Advisory Committee Meeting, DC. PI Daszak presented results from this study as part of an invited talk.
• Consortium of Universities for Global Health Conf., Washington DC, 2014. PI Daszak presented data from this study in a session on disease ecology

3) Public outreach
• PI Daszak reported on this project at an EcoHealth Alliance meeting hosted by the Cosmos Club, 2014

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces. Early in Year 2 of the study, it is anticipated that all of the qualitative research (i.e, 5-7 focus groups and ~100 ethnographic interviews) will be completed, transcribed and translated. It is anticipated that a total of approximately 100 ethnographic interviews and five to seven focus groups will be conducted in targeted areas with known bat populations in Yunnan, Guangxi, Guangdong and Fujian over the next few months. At least one of the focus groups and an estimated 35-40% of the interviews and surveys will be conducted with women. Subjects are enrolled in this study without regard to ethnicity.
Preliminary analyses will be conducted and will focus on the factors least understood, but crucial to the development of a behavioral risk survey that captures relevant behaviors and practices. Factors include specific human-animal interactions, experiences of unusual illness in both humans and animals, and an assessment of the context within which these activities occur. Because of the unique dataset and the expected richness of the data, additional research questions will be developed and explored using grounded theory, as well as more recently developed methods such as narrative analysis and case oriented understanding.

Results from preliminary analyses will contribute to the development of the behavioral risk survey. A behavioral survey sampling frame and recruitment materials are currently being developed. After pilot testing the behavioral survey, we will begin concurrent biologic specimen collection from bats, other wildlife and humans to compare circulating CoV strains in the bat population with serological exposure in human populations. The behavioral risk survey will facilitate the identification of explicit behavioral risks and practices that are found among study participants seropositive for SARS-like corona virus. These findings will be used to develop better risk mitigation policies and targeted intervention strategies.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk.
Future steps to optimize the model of role of species diversity in CoV emergence risk will include:
1. Parameterizing with actual data on species diversity and abundance of animals from Southern China markets.
2. Parameterizing with species-specific data on CoV prevalence and strain variation in different bat species from field surveillance, e.g. if Rhinolopus spp. represent the highest risk for SARS-related CoV emergence, these species will be given a higher weight.
3. Incorporation of CoV lineage specific probabilities for inter-host spillover based on receptor binding data.

We will also conduct further modeling activities, including:
1. Comparative cophylogenetic analyses of bat host and CoV RdRp and Spike gene phylogenies, to assess patterns of evolutionary congruence and frequency of cross-species transmission.
a. Using previously published data from literature and Genbank
b. Using sequence data from our S. China surveillance
2. Calculate CoV divergence times using Spike RBD sequences for S. China.
3. Construct initial generalized linear mixed model to predict CoV diversity using S. China data and bat host-specific trait data. Update model regularly with new data from CoV screening in different bat species.

Specific Aim 3: Testing predictions of CoV inter-species transmission.
The following experiments will be undertaken in Year 2:
1. Animal infection experiment with SARS-like CoV
Option 1. Virus infection through ACE2 humanized mouse. Human ACE2 promotor (9-10 kb) and ACE2 will be inserted into a expressing vector and sent to a commercial company to generate transgenic mice. The stably expressed human ACE2 mice will be used for virus infection.
Option 2. Virus infection through SARS-CoV susceptible animals such as ferrets.
All above animal infection experiment will be performed under the containment of BSL3.
2. Continued surveillances of SARS-like CoVs in Yunnan and Guangdong provinces and isolation of novel virus strains.
3. Surveillance of infection in human populations by SARS-like CoVs. This work will be performed at two locations, one each in Yunnan and Guangdong provinces. PCR and ELISA will be used, respectively, for detection of viral replicase gene and antibody against the viral

nucleocapsid protein.

Daszak, Peter, PI

Year 1 Report for Understanding the Risk of Bat Coronavirus Emergence

Award Number: 1R01AI110964-01

B2: What was accomplished under these goals?

<u>Specific Aim 1</u>: Assessment of CoV spillover potential at high risk human-wildlife interfaces.

In the first year of this R01, we have:

1) Designed a behavioral risk study using an iterative approach that begins with rapid and focused qualitative research at or near biological surveillance sites in China where bats have previously been captured, sampled and found to contain novel CoVs. The study design includes: 1) structured observation and mapping of public spaces, 2) focus groups and 3) ethnographic interviews. The primary enrollment criteria are related to occupational exposure to bats and residence near bats. This research is conducted with two groups of individuals: those involved in the bat value chain (from hunter through market to consumer) and those highly exposed to bats (e.g., cave dwellers). The qualitative data will be used to inform a behavioral risk survey, as well as to contextualize findings from behavioral surveillance analyses.

2) Conducted observational research and mapping in: ***Yunnan***: In and around Xiang Yun village (two clinics and one wildlife restaurant); in and around the remote Lu Feng village (1 wildlife farm, 1 wildlife butcher and 1 wildlife restaurant) and at the An Ning communicable disease hospital complex; ***Guangxi***: In and around LiPu, (two markets, 3 wildlife farms, 1 wildlife restaurant); and ***Guangdong***: Guangzhou wildlife market, Foshon wildlife market (this market is where the first cases of SARS were traced back to in 2003).

3) Secured local IRB approval in November 2014 from Wuhan University School of Public Health, Hubei Province, to conduct qualitative research, to administer behavioral surveys and to collect biological data including blood (no more than 550ml), sputum, and stool samples from humans. We secured US IRB approval through Hummingbird IRB (2014-23 approval letter sent to NIH) in November 2014 for qualitative, quantitative and biological specimen data collection.

4) Drafted protocols, guides, and training modules for Observational Research, Focus Groups, and Ethnographic Interviews and pilot tested these. The Observational Guide and Ethnographic Interview materials were pilot tested in live animal markets in Queens, New York City. Consistent with the original proposal, we have trained interviewers and identified key informants. Key informants include community health workers from three different administrative level CDCs, Barefoot Doctors, public health clinicians, local wildlife farmers and wildlife restaurant owners, as well as market vendors and workers. Ethnographic and Focus Group Interviews to be conducted pending NIH approval of IRB approval letter.

<u>Specific Aim 2</u>: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk.

1) *Collation and preliminary analysis of published bat Coronavirus data to optimized specimen collection and taxonomic targets for surveillance.*

Over the last decade a large number of bat viral discovery studies have been published globally (including a large number focused on CoVs). In year 1, we conducted the first ever systematic analysis of these data. We collated literature from over 100 viral discovery studies in bats, to examine patterns of host range and known viral diversity in different bat taxa (Young and Olival, In Review). We found that Coronavirus diversity has been most thoroughly characterized in a few bat families, including the Vespertillionidae and 5 other families, but several bat taxa remain under-represented in global virus surveillance efforts (**Fig 1**). Identification of these surveillance gaps allows us to better target our field surveillance towards bat taxa where CoV diversity is largely unknown (blue and light colored cells, **Fig 1**). These analyses were completed at various taxonomic levels, including by bat subfamily and genera (Family level analysis only shown).

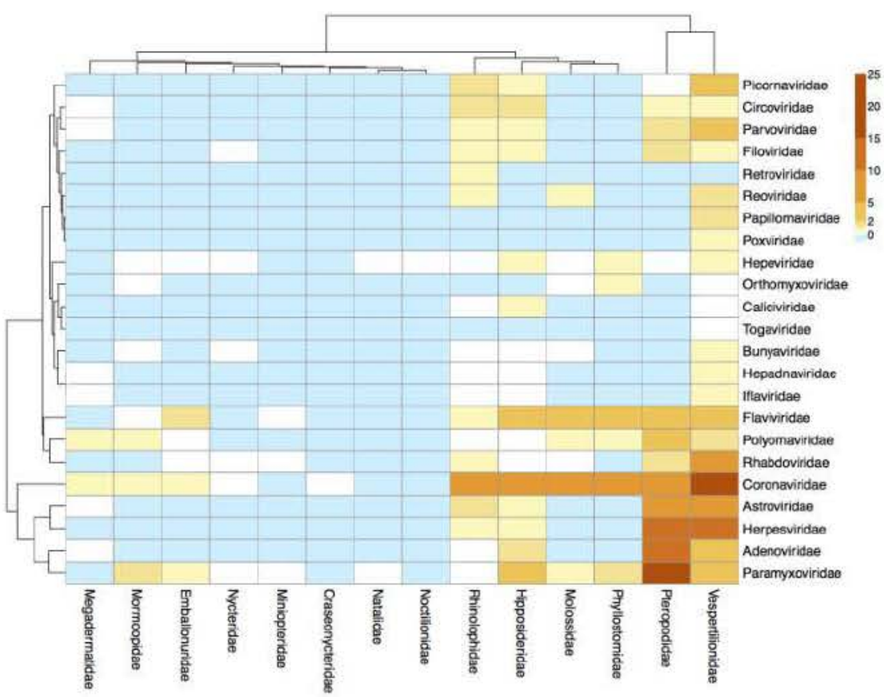


Figure 1. Heat map of viral richness by bat host and viral family, clustered by similarity in viral richness across host and viral families.

To maximize our chances of discovering CoVs, we need to define the number of specimens required for our bat surveillance work and the bat taxonomic groups on which to focus our surveillance. We used generalized linear mixed models (GLMM) and applied this to a subset of our collated data for CoVs alone. We found that sample type screened (feces), collection methods, and the number of specimens tested best explains the probability of finding an individual CoV positive sample. We will now use these

approaches to increase the likelihood of getting positive samples in our fieldwork in China.

2) *Preliminary 'What-if' Model: Role of species diversity in CoV emergence risk.* We built a mathematical model to analyze different scenarios of CoV spillover. We began with an assessment of how the diversity of wildlife (and other factors) in wet markets may affect the probability of CoV zoonotic spillover. We modeled evolution of CoVs within wildlife in a market following the initial introduction of a novel virus in one specific host. We assume this initial virus is a single genotype that does not yet have a great enough rate of spread to create an epidemic, but has a rate of spread close to this threshold. When this virus infects a new host, a new genotype is generated, based on random drift from the infecting genotype. We use Neutral Theory of Species Diversity to specify the species distribution in the market, for a given total number of species and total abundance of animals. We assume 500 animals in the market, and alter the species diversity from 3 to over 40. These numbers are easily attained in a small to medium market in Southern China (and in year 2 we will groundtruth these assumptions)

As the number of species present in a market increases from 3 to 20, the percent of simulations where zoonotic spillover occurred from any of the animals into humans increases (**Fig 2**). However, the risk remains fairly level if wildlife biodiversity increases above that level. The probability of epidemic failure is inverse to the probability of a zoonotic spillover taking hold and decreases with increasing species diversity (**Fig 2**). Therefore our null model shows that reducing the diversity of species in live animal markets could reduce the risk of zoonotic spillover, including of potentially pandemic CoVs.

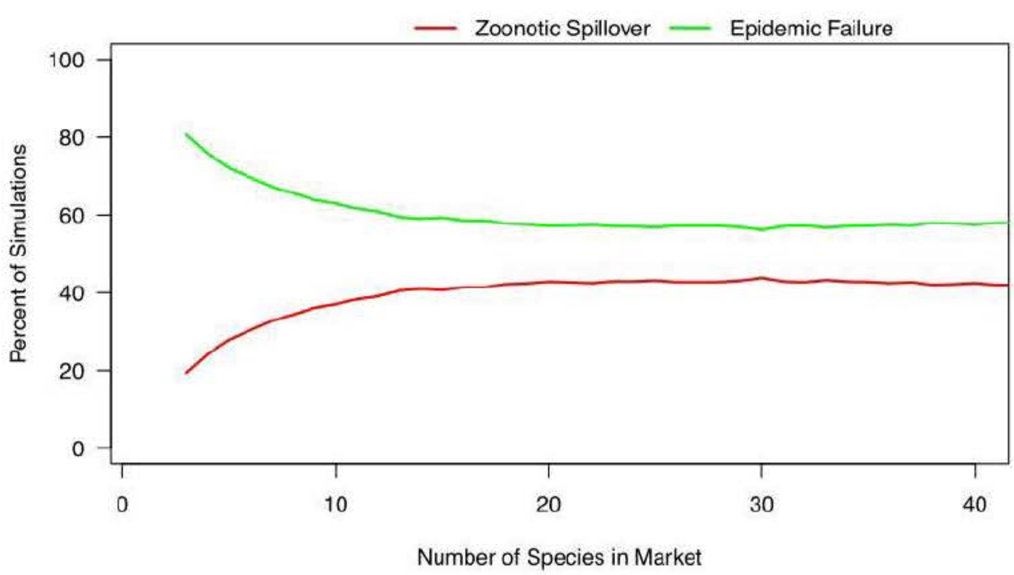


Figure 2. 'What-if' scenario model based on the Neutral Theory of Species Diversity to examine the role of wildlife species diversity for CoV spillover in markets.

Specific Aim 3: Testing predictions of CoV inter-species transmission.

1) Bat Coronavirus Surveillance in 2014

We collected 1555 anal swab samples, 1357 fecal samples, 461 blood samples, 469 serum samples and 24 tissue samples from > 14 bat genera in 5 provinces and in Laos (**Table 1**).

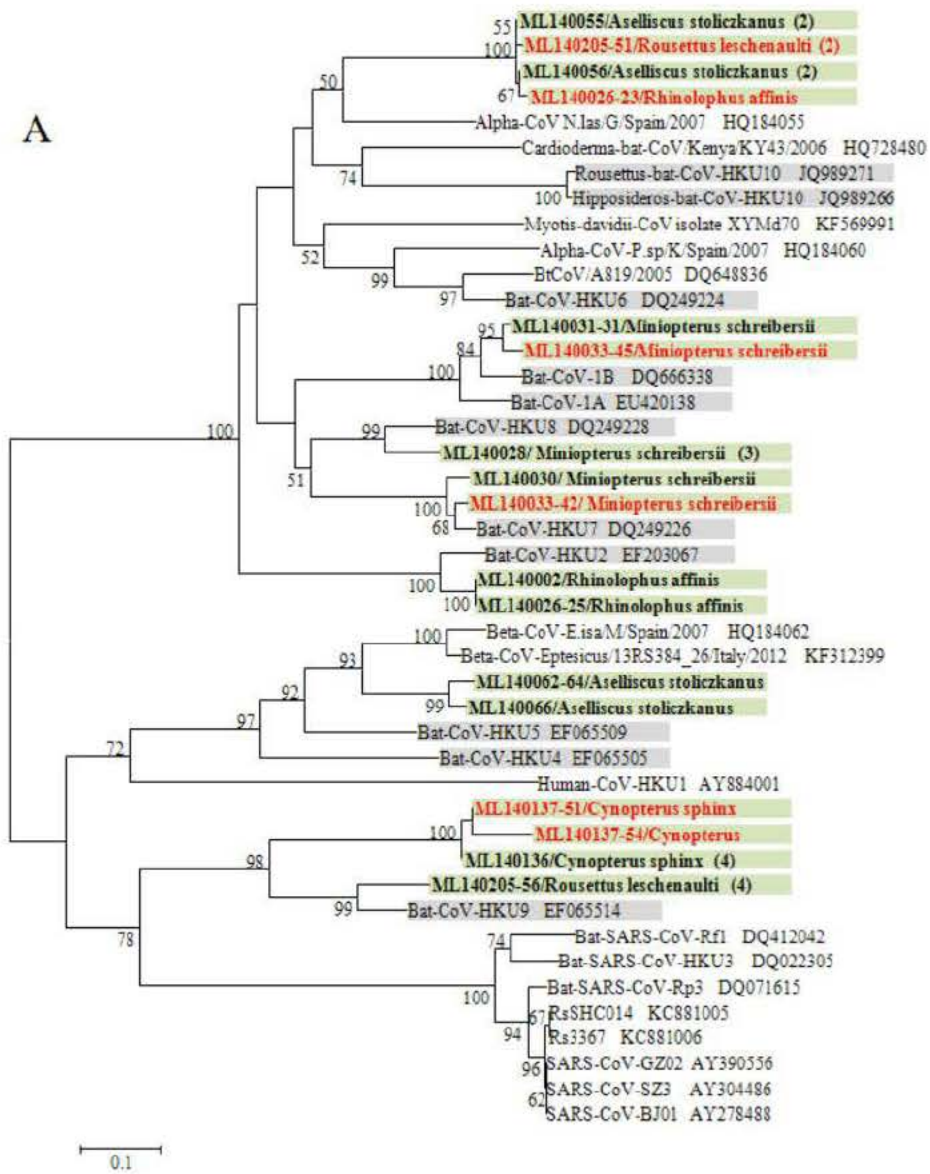
Table 1 Bat Samples collected for CoV surveillance in 2014

		Anal	Oral	Fecal	Blood	Serum	tissue
Jan. 2014	Mengla, Yunnan	164	--	--	--	--	--
Mar. 2014	Beihai, Guangxi	30	--	--	--	--	--
Apirl 2014	Shenzhen	77	--	--	--	--	--
May 2014	Ruyuan, Guangdong	167	--	--	--	--	--
	Chuxiong, Yunnan	52	52	103	--	8	16
	Jinning, Yunnan	--	--	131	--	--	--
	Mojiang, Yunnan	25	25	103	--	--	3
May-Sep. 2014	Xianning, Hubei	--	--	583	--	--	--
Jun. 2014	Guangdong	77	--	--	--	--	--
Jul. 2014	Hainan	460	--	--	--	--	--
Aug. 2014	Yichang, Hubei	--	--	114	--	--	--
Sep. 2014	Guilin,Guangxi	121	122	--	122	122	--
	Guangdong	335	337	--	335	335	--
Jul.--Sep. 2014	Mojiang, Yunan	--	--	96	--	--	--
Oct. 2014	Jinning, Yunan	13	13	6	3	3	4

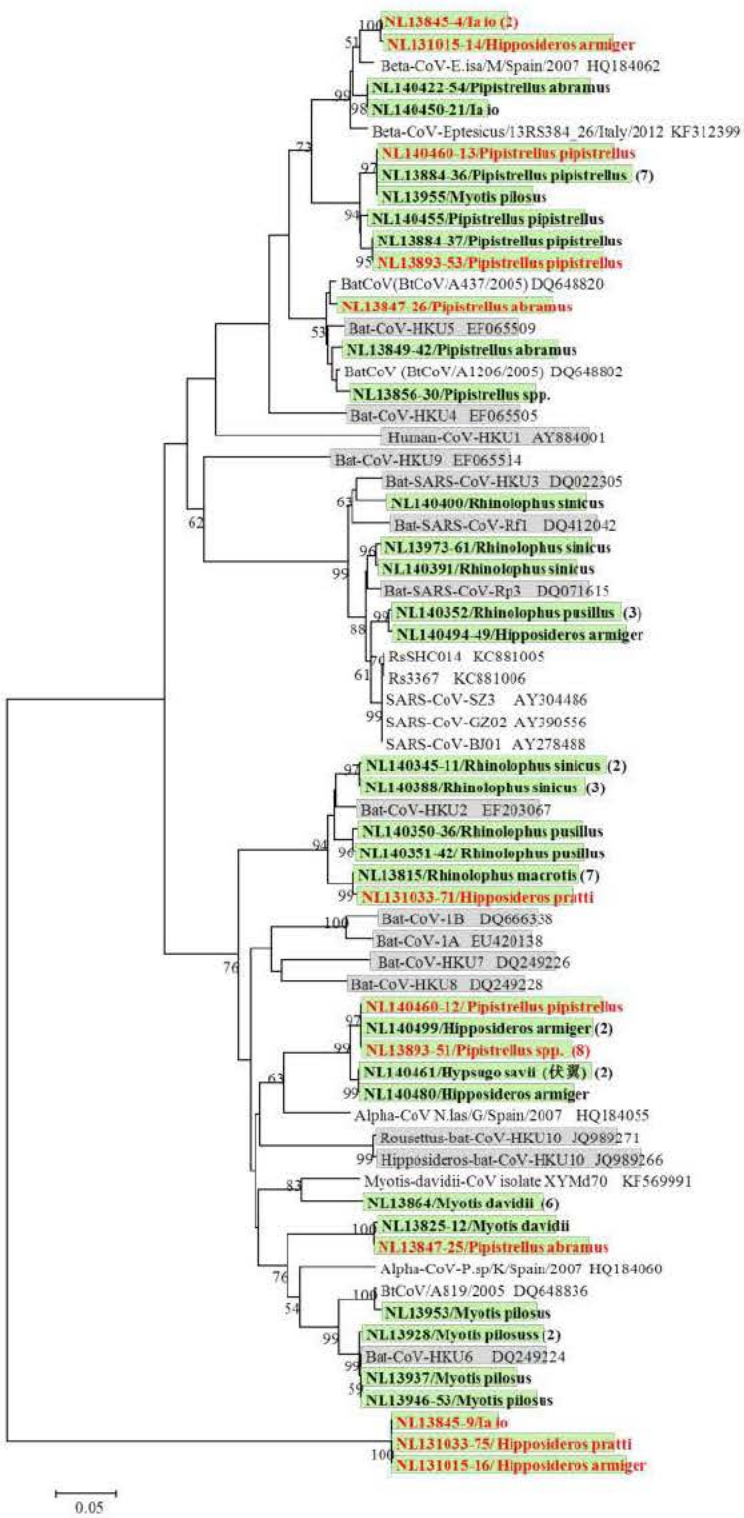
	Mojiang, Yunan	34	34	100	1	1	1
	Laos			121			
	Total	1555	583	1357	461	469	24

CoV was detected in 14% (336/2329) samples (**Table 2**). Diverse alphacoronaviruses were identified, including isolates closely related to Bat CoV 1A, 1B, HKU2, HKU6, HKU7, HKU8 and HKU10. Groups of novel alphacoronaviruses were discovered in a variety of bat species (**Fig 3**). **Novel SARS-like coronaviruses were detected in *Rhinolophus* bats collected in different regions of Guangdong province.** Diverse novel betacoronaviruses related to HKU5 were detected in *Pipistrellus* bats and *Ia io* in Guangdong and in *Aselliscus stoliczkanus* in Mengla, Yunnan. Novel coronaviruses related to HKU9 were found in *Cynopterus sphinx* and *Rousettus leschenaulti* in Mengla (**Fig 3A**). In addition, sequences significantly divergent to other CoV were obtained from three samples of *Ia io* and *Hipposideros* bats.

A


ML140055/Aselliscus stoliczkanus (2)
ML140205-51/Rousettus leschenaulti (2)
ML140056/Aselliscus stoliczkanus (2)
ML140026-23/Rhinolophus affinis
Alpha-CoV N.las/G/Spain/2007 HQ184055
Cardioderma-bat-CoV/Kenya/KY43/2006 HQ728480
Rousettus-bat-CoV-HKU10 JQ989271
Hipposideros-bat-CoV-HKU10 JQ989266
Myotis-davidii-CoV isolate XYMd70 KF569991
Alpha-CoV-P.sp/K/Spain/2007 HQ184060
BtCoV/A819/2005 DQ648836
Bat-CoV-HKU6 DQ249224
ML140031-31/Miniopterus schreibersii
ML140033-45/Miniopterus schreibersii
Bat-CoV-1B DQ666338
Bat-CoV-1A EU420138
Bat-CoV-HKU8 DQ249228
ML140028/ Miniopterus schreibersii (3)
ML140030/ Miniopterus schreibersii
ML140033-42/ Miniopterus schreibersii
Bat-CoV-HKU7 DQ249226
Bat-CoV-HKU2 EF203067
ML140002/Rhinolophus affinis
ML140026-25/Rhinolophus affinis
Beta-CoV-E.isa/M/Spain/2007 HQ184062
Beta-CoV-Eptesicus/13RS384_26/Italy/2012 KF312399
ML140062-64/Aselliscus stoliczkanus
ML140066/Aselliscus stoliczkanus
Bat-CoV-HKU5 EF065509
Bat-CoV-HKU4 EF065505
Human-CoV-HKU1 AY884001
ML140137-51/Cynopterus sphinx
ML140137-54/Cynopterus
ML140136/Cynopterus sphinx (4)
ML140205-56/Rousettus leschenaulti (4)
Bat-CoV-HKU9 EF065514
Bat-SARS-CoV-Rf1 DQ412042
Bat-SARS-CoV-HKU3 DQ022305
Bat-SARS-CoV-Rp3 DQ071615
RsSHC014 KC881005
Rs3367 KC881006
SARS-CoV-GZ02 AY390556
SARS-CoV-SZ3 AY304486
SARS-CoV-BJ01 AY278488
0.1

B


NL13845-4/Ia io (2)
NL131015-14/Hipposideros armiger
Beta-CoV-E.isa/M/Spain/2007 HQ184062
NL140422-54/Pipistrellus abramus
NL140450-21/Ia io
Beta-CoV-Eptesicus/13RS384_26/Italy/2012 KF312399
NL140460-13/Pipistrellus pipistrellus
NL13884-36/Pipistrellus pipistrellus (7)
NL13955/Myotis pilosus
NL140455/Pipistrellus pipistrellus
NL13884-37/Pipistrellus pipistrellus
NL13893-53/Pipistrellus pipistrellus
BatCoV(BtCoV/A437/2005) DQ648820
NL13847-26/Pipistrellus abramus
Bat-CoV-HKU5 EF065509
NL13849-42/Pipistrellus abramus
BatCoV (BtCoV/A1206/2005) DQ648802
NL13856-30/Pipistrellus spp.
Bat-CoV-HKU4 EF065505
Human-CoV-HKU1 AY884001
Bat-CoV-HKU9 EF065514
Bat-SARS-CoV-HKU3 DQ022305
NL140400/Rhinolophus sinicus
Bat-SARS-CoV-Rf1 DQ412042
NL13973-61/Rhinolophus sinicus
NL140391/Rhinolophus sinicus
Bat-SARS-CoV-Rp3 DQ071615
NL140352/Rhinolophus pusillus (3)
NL140494-49/Hipposideros armiger
RsSHC014 KC881005
Rs3367 KC881006
SARS-CoV-SZ3 AY304486
SARS-CoV-GZ02 AY390556
SARS-CoV-BJ01 AY278488
NL140345-11/Rhinolophus sinicus (2)
NL140388/Rhinolophus sinicus (3)
Bat-CoV-HKU2 EF203067
NL140350-36/Rhinolophus pusillus
NL140351-42/ Rhinolophus pusillus
NL13815/Rhinolophus macrotis (7)
NL131033-71/Hipposideros pratti
Bat-CoV-1B DQ666338
Bat-CoV-1A EU420138
Bat-CoV-HKU7 DQ249226
Bat-CoV-HKU8 DQ249228
NL140460-12/ Pipistrellus pipistrellus
NL140499/Hipposideros armiger (2)
NL13893-51/Pipistrellus spp. (8)
NL140461/Hypsugo savii (伏翼) (2)
NL140480/Hipposideros armiger
Alpha-CoV N.las/G/Spain/2007 HQ184055
Rousettus-bat-CoV-HKU10 JQ989271
Hipposideros-bat-CoV-HKU10 JQ989266
Myotis-davidii-CoV isolate XYMd70 KF569991
NL13864/Myotis davidii (6)
NL13825-12/Myotis davidii
NL13847-25/Pipistrellus abramus
Alpha-CoV-P.sp/K/Spain/2007 HQ184060
BtCoV/A819/2005 DQ648836
NL13953/Myotis pilosus
NL13928/Myotis pilosuss (2)
Bat-CoV-HKU6 DQ249224
NL13937/Myotis pilosus
NL13946-53/Myotis pilosus
NL13845-9/Ia io
NL131033-75/ Hipposideros pratti
NL131015-16/ Hipposideros armiger
0.05

C

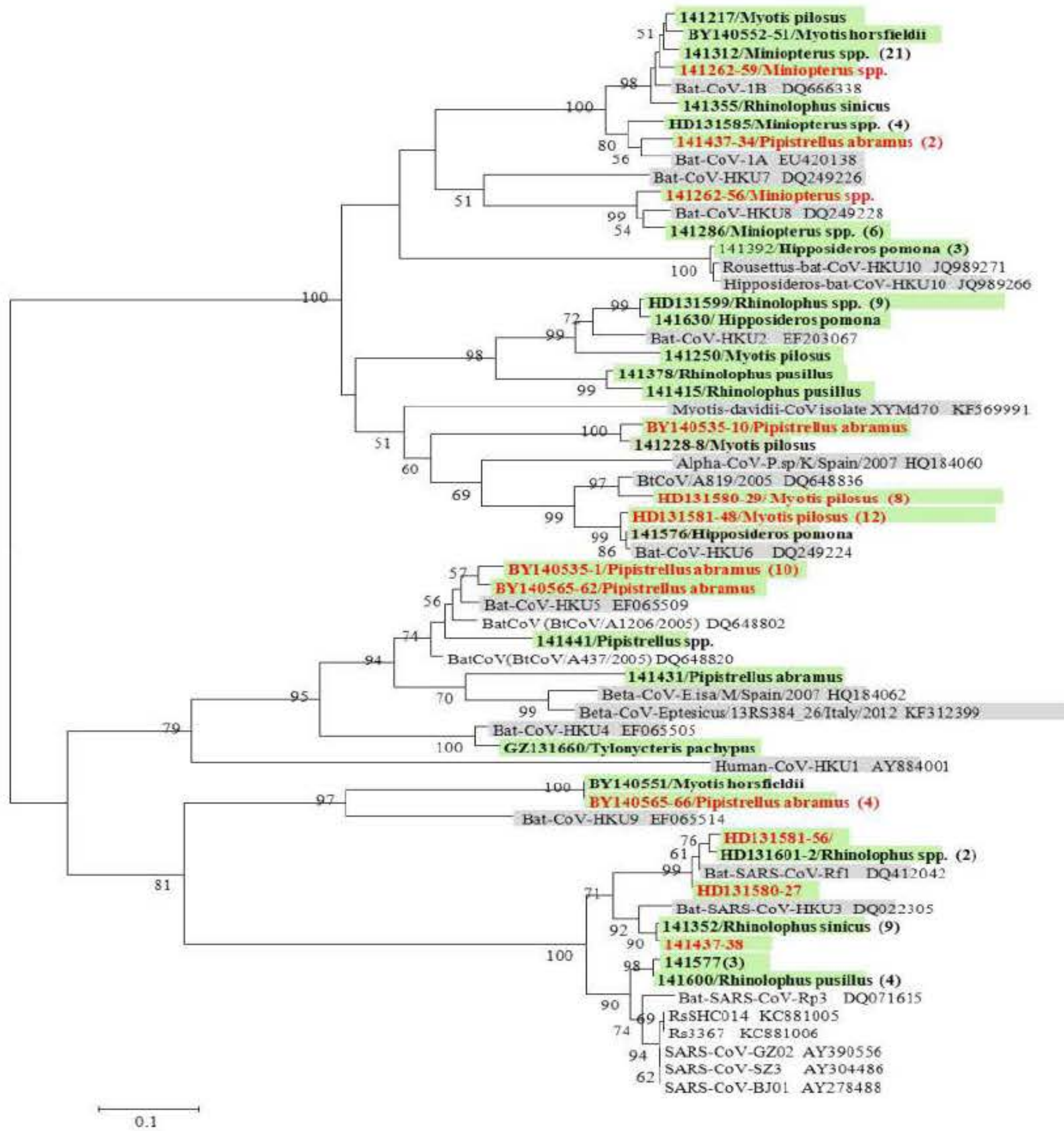


Figure 3: Phylogenetic analysis of partial RdRp gene of CoV. CoVs identified in this study are in bold and named by the sample numbers. Sequence amplified from samples co-infected with two CoV strains are indicated in red. (A) CoVs detected in Mengla, Yunnan. (B) CoVs detected in Ruyuan, Guangdong. (C) CoVs detected in other regions in Guangdong.

2) Complete S gene sequencing and recombination analysis of novel SARS-like CoV

We amplified the full-length S gene of the novel SL-CoV detected in a *Rhinolophus sinicus* colony in Yunnan Province. In addition to our previously reported Rs3367 and RsSHC014, we now have 24 new full-length S gene sequences from 22 samples. Phylogenetic analysis showed that these SL-CoV are diverse, **and identified two strains of novel SL-CoV more closely related to SARS-CoV than Rs3367** (**Fig 4A**). Our new strains named Rs4841 and Rs4874 share the highest homology to SARS-CoV than any other known SL-CoV, including those we published previously in *Nature*. These viruses are highly similar to SARS-CoV in receptor-binding domain (RBD) sequence but also in N-terminal domain (NTD) (**Figure 4B**). Analysis of the complete S protein shows > 97% amino acid identify to that of SARS-CoV isolates.

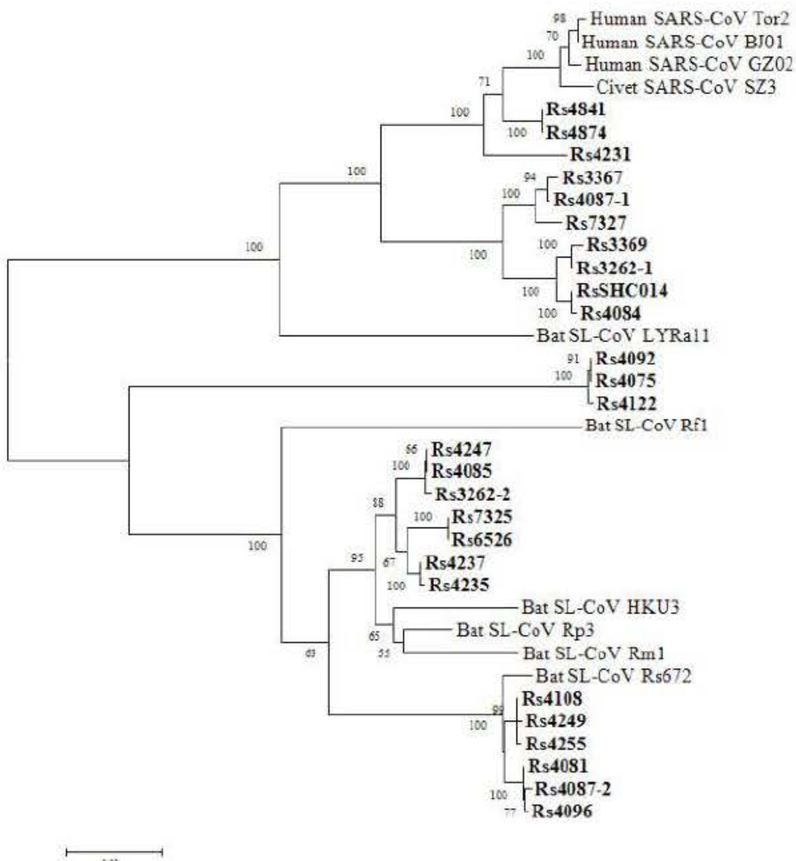


Figure 4A Phylogenetic analysis of novel SL-CoVs discovered in Year 1 of this project (**Bold**), based on amino acid sequences of complete S gene.

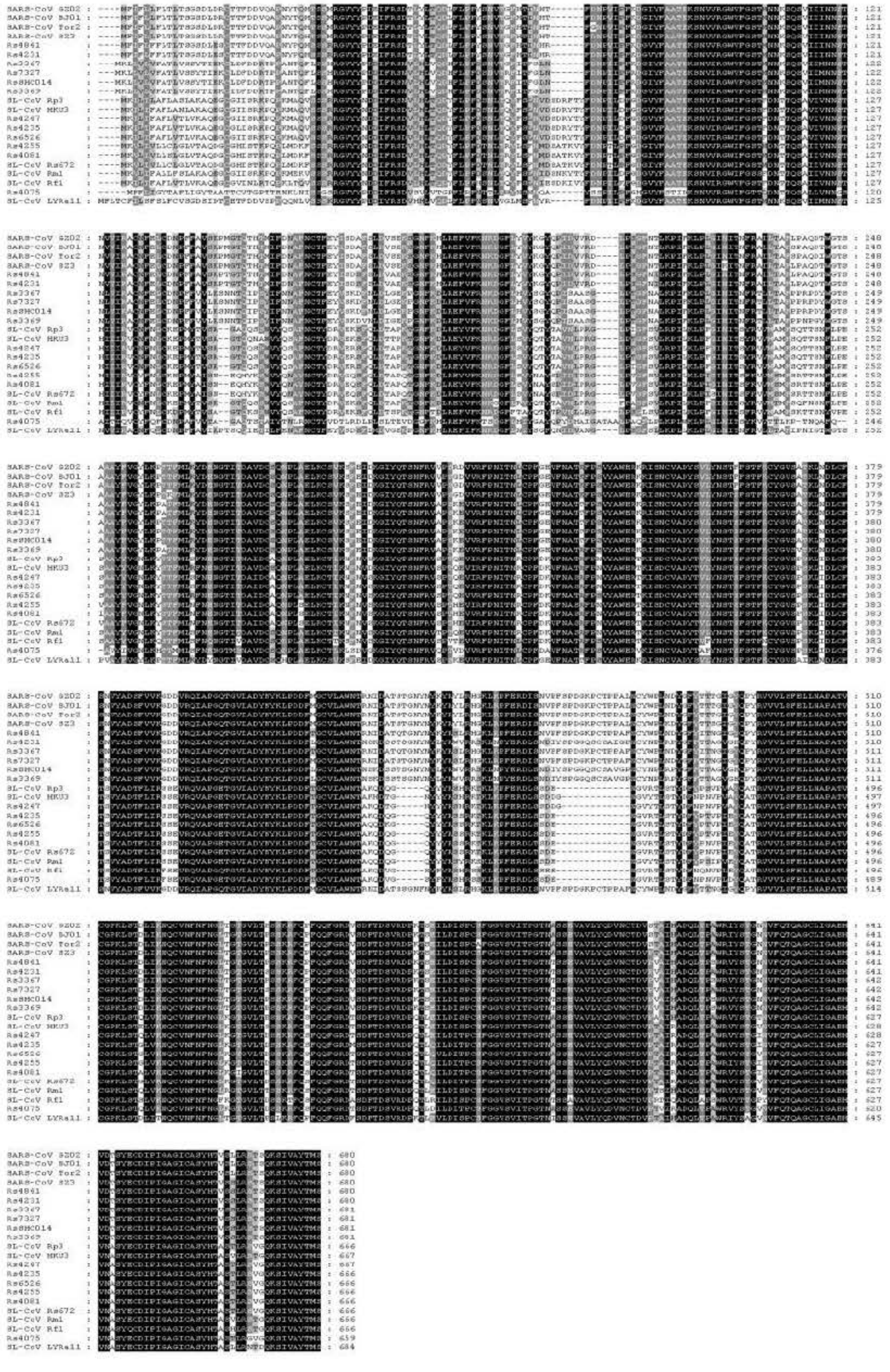


Figure 4B Alignment of amino acid sequences of S1 (aa1-680) of SARS-CoV and bat SL-CoVs.

We performed recombination analysis and detected potential recombination events in S genes of multiple SL-CoV strains suggesting that that the region around nt1000 in RBD is a recombination hotspot. In addition, a novel SL-CoV strain (Rs4075) with an NTD sequence distinct from all other SL-CoVs was identified (**Figure 4**). The results suggest that the high genetic diversity of SL-CoV in this colony is related to the frequent recombination.

3) Virus isolation and characterization

Isolation on Vero E6 cells was conducted on all CoV PCR-positive samples using an optimized protocol. Repoducible CPE was observed for Rs4841 (the strain closely related to SARS-CoV in both the RBD and NTD region of the S protein). Purified virions displayed typical coronavirus morphology under electron microscopy, and this novel isolate was named SL-CoV-WIV16.

We conducted virus infectivity studies (using HeLa cells expressing or not expressing ACE2 from humans, civets or Chinese horseshoe bats) to determine whether SL-CoV-WIV16 can use ACE2 as a cellular entry receptor **(Figure 5).** We found that WIV16 is able to use ACE2 of different origins as an entry receptor.

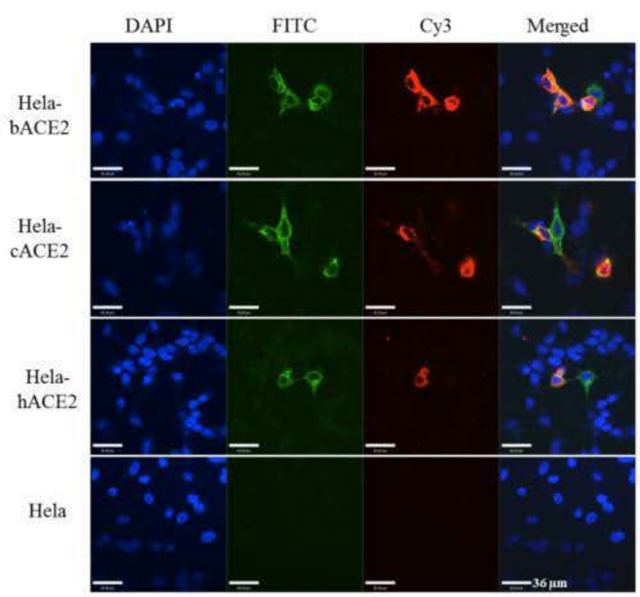


Figure 5. Analysis of receptor usage of SL-WIV16 determined by immunofluorescence assay. Determination of virus infectivity in Hela cells without the expression of ACE2. b, bat; c, civet; h, human. Nuclei are stained with DAPI. The columns (from left to right) show staining of nuclei (blue), ACE2 expression (green), virus replication (red) and merged triple-stained images.

To assess its cross-species transmission potential, we conducted infectivity assays in cell lines from a range of species. Our results (**Figure 6)** show that SL-CoV-WIV16 can grow in human alveolar basal epithelial (A549), pig kidney-15 (PK15), *Rhinolophus sinicus* kidney (RSKT), *Macaca mulatta* Kidney cell lines (MK2) and human lung carcinoma (NCI-H292), but not in human cervix (HeLa), Syrian golden hamster kidney (BHK21), *Myotis davidii* kidney (BK), *Myotis davidii* intestine (MDI), *Rousettus leschenaulti* kidney (RLK), *Rhinolophus sinicus* brain (RSBT), *Rhinolophus sinicus* heart (RSHT), *Rhinolophus sinicus* Lung (RSLuT), *Rhinolophus sinicus* intestine (RSI) or *Pteropus alecto* kidney (PaKi) lines.

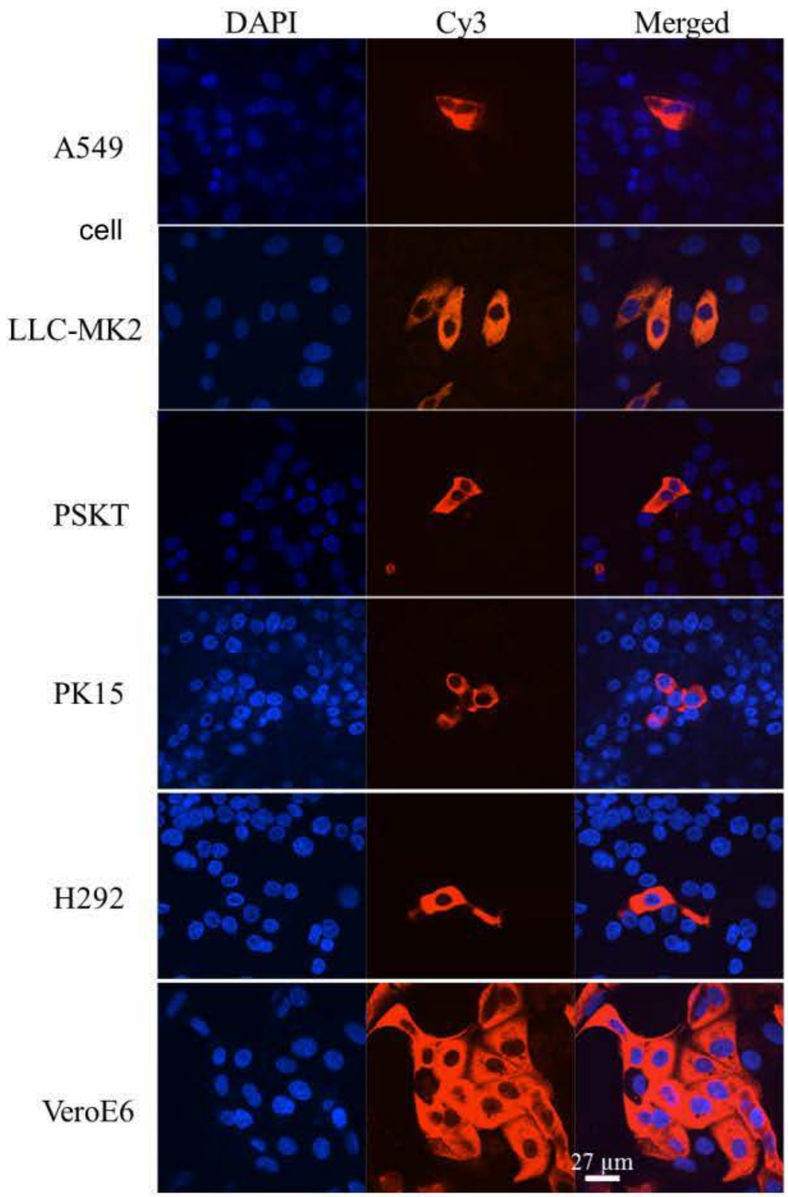


Figure 6 Cell infection with SL-CoV WIV16 determined by immunofluorescence assay with antibody against SARS-like coronavirus nucleocapsid protein. The columns (from left to right) show staining of nuclei (blue), virus replication (red) and merged double-stained images.

Daszak, Peter, PI

Accomplishments for Understanding the Risk of Bat Coronavirus Emergence

Grant Number 5R01AI110964

B4: Opportunities for Training and Professional Development

In year 1 of this work, we trained undergraduate interns from Columbia University in modeling approaches to understand bat risk of harboring zoonotic CoVs. In the behavioral risk work, we used standardized training materials for all three qualitative behavioral risk data collection methodologies have been created. Materials were used to train six people in New York City and 12 people in Yunnan, China, of which 11 were from three different administrative levels of local government Centers for Disease Control (CDC). The trainees include the Chinese EcoHealth Alliance Field Coordinator and Yunnan Provincial CDC personnel: six researchers from Xiangyun County CDC (4 women, 2 men), two from Yunnan Institute for Endemic Diseases (Yunnan Provincial CDC; 2 men), and three from Lu Feng County CDC (3 men).

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
N/A: Not Journal	Olival KJ, Weekley CC, Daszak P. Bats and Viruses. Wang L editor. New York: John Wiley & Sons, Inc.; 2015. What we know and need to know
Non-Compliant	(b) (4) [redacted]
PMC Journal - In process	(b) (4) [redacted]

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
(b) (6)	Y	DASZAK, PETER	(b) (6)	(b) (6)	BS,PHD	PD/PI			(b) (4), (b) (6)			NA
	Y	KE, CHANGWEN			PHD	Co-Investigator				CDC and Prevention of Guangdong Province	CHINA	NA
	Y	ZHANG, YUNZHI		(b) (6)	PHD	Co-Investigator				Yunnan Institute of Endemic Diseases Control & Prevention	CHINA	NA
	Y	ZHU, GUANGJIAN		(b) (6)	PHD	Co-Investigator				East China Normal University	CHINA	NA
(b) (6)	Y	SHI, ZHENGLI		(b) (6)	PhD	Co-Investigator				Wuhan Institute of Virology	CHINA	NA
(b) (6)	N	CHMURA, ALEKSEI A	(b) (6)	(b) (6)	BS	Non-Student Research Assistant						NA
(b) (6)	Y	OLIVAL, KEVIN J	(b) (6)	(b) (6)	PHD	Co-Investigator						NA
(b) (6)	Y	HOSSEINI, PARVIEZ RANA	(b) (6)	(b) (6)	BS,PHD	Co-Investigator						NA
(b) (6)	Y	ZHANG, SHUYI		(b) (6)	PHD	Co-Investigator				East China Normal University	CHINA	NA
	Y	GE, XINGYI			PHD	Co-Investigator				Wuhan Institute of Virology	CHINA	NA
(b) (6)	Y	EPSTEIN, JONATHAN H	(b) (6)	(b) (6)	MPH,DVM,BA,PHD	Co-Investigator						NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement

Aca - Person Months (Academic) Sum - Person Months (Summer)	OT - Other NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

No

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Dollar Amount	Country
50902	CHINA

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

Yes

Is the research exempt from Federal regulations?

No

Does this project involve a clinical trial?

No

G.4.b Inclusion Enrollment Data

Report Attached: Understanding the Risk of Bat Coronavirus Emergence-PROTOCOL-001

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

No

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

Yes

As reported by Dr. Peter Daszak (PI) to NIH in May 2014, all of the following senior/key/other personnel were enrolled in and passed the Human Subjects Research Course provided by the Collaborative Institutional Training Initiative (CITI Program) at the University of Miami (http://citiprogram.org). The CITI Program is a leading provider of research education content with web based training materials serving millions of learners at academic institutions, government agencies, and commercial organizations in the U.S. and around the world.

Peter Daszak, PI
Zhengli Shi, Co-Investigator
Shuyi Zhang, Co-Investigator
Changwen Ke, Co-Investigator
Jonathan Epstein, Co-Investigator
Kevin Olival, Co-Investigator
Parviez Hosseini, Co-Investigator
Xingyi Ge, Co-Investigator
Guangjian Zhu, Co-Investigator
Yunzhi Zhang, Co-Investigator
Aleksei Chmura, Program Coordinator

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: EcoHealth Alliance, Inc.	077090066	NY-010	460 West 34th Street 17th Floor New York NY 100012317
Wuhan Institute of Virology	529027474		Xiao Hong Shan, No. 44 Wuchang District Wuhan
East China Normal University	420945495		3663 Zhongshan Beilu Shanghai

G.9 FOREIGN COMPONENT

Organization Name: East China Normal University
Country: CHINA
Description of Foreign Component:
Institution of Co-Investigators Dr. Shuyi Zhang and Dr. Guangjian Zhu

Organization Name: Wuhan Institute of Virology
Country: CHINA
Description of Foreign Component:
Primary Laboratory and Institute of Co-Investigators Dr. Zhengli Shi and Dr. Xingyi Ge

Organization Name: Yunnan Institute of Endemic Diseases Control and Prevention
Country: CHINA
Description of Foreign Component:
Institution of Co-Investigator Dr. Yunzhi Zhang

Organization Name: Center for Disease Control and Prevention of Guangdong
Country: CHINA
Description of Foreign Component:
Institution of Co-Investigator Dr. Changwen Ke

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

Inclusion Enrollment Report

Inclusion Data Record (IDR) #: 166195

Study Title: Understanding the Risk of Bat Coronavirus Emergence-PROTOCOL-001

Foreign/Domestic: Foreign

Planned Enrollment Report

Planned Enrollment Total: 2,460

NOTE: Planned enrollment data exists in the previous format; the PD/PI did not enter the planned enrollment information in the modified format and was not required to do so. Only the total can be provided.

Cumulative Enrollment Report

NOTE: No cumulative inclusion enrollment data exists in the previous inclusion format or modified format. Although prompted to do so, the PD/PI did not enter information in the modified format. No data can be provided.

RESEARCH **Federal Award Date:** 05/05/2017
Department of Health and Human Services
National Institutes of Health

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI110964-03 REVISED
FAIN: R01AI110964

Principal Investigator(s):
PETER DASZAK, PHD

Project Title: Understanding the Risk of Bat Coronavirus Emergence

Aleksei Chmura
President
460 West 34th Street
17th Floor
New York, NY 100012317

Award e-mailed to: [redacted] (b) (6)

Period Of Performance:
Budget Period: 06/01/2016 – 05/31/2017
Project Period: 06/01/2014 – 05/31/2019

Dear Business Official:

The National Institutes of Health hereby revises this award (see “Award Calculation” in Section I and “Terms and Conditions” in Section III) to ECOHEALTH ALLIANCE, INC. in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the “Terms and Conditions” is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as “Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI110964. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.” Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator’s Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Philip E. Smith
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI110964-03 REVISED

Award Calculation (U.S. Dollars)

Salaries and Wages	$167,708
Fringe Benefits	$54,168
Personnel Costs (Subtotal)	$221,876
Materials & Supplies	$7,250
Travel	$35,918
Other	$11,050
Subawards/Consortium/Contractual Costs	$213,239
Federal Direct Costs	$489,333
Federal F&A Costs	$121,757
Approved Budget	$611,090
Total Amount of Federal Funds Obligated (Federal Share)	$611,090
TOTAL FEDERAL AWARD AMOUNT	$611,090
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$0

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
3	$611,090	$611,090
4	$597,112	$597,112
5	$581,646	$581,646

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name: Allergy and Infectious Diseases Research
CFDA Number: 93.855
EIN: 1311726494A1
Document Number: RAI110964A
PMS Account Type: P (Subaccount)
Fiscal Year: 2016

IC	CAN	2016	2017	2018
AI	8472350	$611,090	$597,112	$581,646

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M51C / **OC**: 414E / **Released**: (b) (6) 05/05/2017
Award Processed: 05/05/2017 07:00:56 PM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI110964-03 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI110964-03 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants

Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI110964. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.
Treatment of Program Income:
Additional Costs

The Research Performance Progress Report (RPPR), Section G.9 (Foreign component), includes reporting requirements for all research performed outside of the United States. Research conducted at the following site(s) must be reported in your RPPR:

San Pya Clinic, BURMA
Institut Pasteur du Cambodge, CAMBODIA
Primate Research Center at Bogor Agricultural University, INDONESIA
Conservation Medicine, Ltd, MALAYSIA
King Chulalongkorn Memorial Hospital, THAILAND
Hanoi Agricultural University, VIETNAM

****

REVISED AWARD: This Notice of Award is revised to provide approval for collaboration with the **Wuhan University School of Public Health** (**CHINA**) in accordance with the request submitted by Aleksei Chmura, Ecohealth Alliance, Inc. on October 6, 2016.

Supersedes previous Notice of Award dated **7/26/2016**.

****

REVISED AWARD: This Notice of Award is revised to provide approval for collaboration with the **Wuhan University School of Public Health** (**CHINA**) in accordance with the request submitted by Aleksei Chmura, Ecohealth Alliance, Inc. on October 6, 2016.

Supersedes previous Notice of Award dated **7/26/2016**.

****

No funds are provided and no funds can be used to support gain-of-function research covered under the October 17, 2014 White House Announcement (NIH Guide Notice NOT-OD-15-011).

Per the letter dated July 7, 2016 to Mr. Aleksei Chmura at EcoHealth Alliance, should any of the MERS-like or SARS-like chimeras generated under this grant show evidence of enhanced virus growth greater than 1 log over the parental backbone strain you must stop all experiments with these viruses and provide the NIAID Program Officer and Grants Management Specialist, and Wuhan Institute of Virology Institutional Biosafety Committee with the relevant data and information related to these unanticipated outcomes.

*****

This Notice of Award (NoA) includes funds for consortium activity with**:**

- **Wuhan Institute of Virology - CHINA** awarded in the Total Costs amount of **$159,122** (**$147,335** Direct Costs + **$11,787** F&A Costs). Future year commitments are as follows: Year 4 Total Costs: $159,122 and Year 5 Total Costs: $159,122
- **East China Normal University** - **CHINA** awarded in the Total Costs amount of **$54,117** (**$50,108** Direct Costs + **$4,009** F&A Costs). Future year commitments are as follows: Year 4 Total Costs: $42,300 and Year 5 Total Costs: $32,454

Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants.nih.gov/grants/policy/nihgps_2013/nihgps_ch15.htm#_Toc271265264.

The written agreement with the consortium must address the negotiated arrangements for meeting the scientific, administrative, financial and reporting requirements for this grant.

No foreign performance site may be added to this project without prior approval of the National Institute of Allergy and Infectious Diseases.

Although a specific amount has been awarded for each consortium, the grantee retains standard rebudgeting authorities.

*****

This award may include collaborations with and/or between foreign organizations. Please be advised that short term travel visa expenses are an allowable expense on this grant, if justified as critical and necessary for the conduct of the project.

*****

Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

> If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.
>
> If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.
>
> If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:
>
>> Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.
>>
>> If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:
>>
>> - A list of the new and/or additional Agent(s) that will be studied;
>> - A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
>> - The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These

individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Jenny L. Greer
Email: (b) (6) **Phone**: (b) (6) **Fax**: 301-493-0597

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SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI110964-03 REVISED

INSTITUTION: ECOHEALTH ALLIANCE, INC.

Budget	Year 3	Year 4	Year 5
Salaries and Wages	$167,708	$167,708	$167,708
Fringe Benefits	$54,168	$54,168	$54,168
Personnel Costs (Subtotal)	$221,876	$221,876	$221,876
Materials & Supplies	$7,250	$7,000	$3,500
Travel	$35,918	$35,918	$35,918
Other	$11,050	$9,800	$9,400
Subawards/Consortium/Contractual Costs	$213,239	$201,422	$191,576
TOTAL FEDERAL DC	$489,333	$476,016	$462,270
TOTAL FEDERAL F&A	$121,757	$121,096	$119,376
TOTAL COST	$611,090	$597,112	$581,646

Facilities and Administrative Costs	Year 3	Year 4	Year 5
F&A Cost Rate 1	44.1%	44.1%	44.1%
F&A Cost Base 1	$276,094	$274,594	$270,694
F&A Costs 1	$121,757	$121,096	$119,376

A. COVER PAGE

Project Title: Understanding the Risk of Bat Coronavirus Emergence	
Grant Number: 5R01AI110964-03	**Project/Grant Period:** 06/01/2014 - 05/31/2019
Reporting Period: 06/01/2015 - 05/31/2016	**Requested Budget Period:** 06/01/2016 - 05/31/2017
Report Term Frequency: Annual	**Date Submitted:** 05/13/2016
Program Director/Principal Investigator Information: PETER DASZAK , BS PHD **Phone number:** (b) (6) **Email:** (b) (6)	**Recipient Organization:** ECOHEALTH ALLIANCE, INC. ECOHEALTH ALLIANCE, INC. 460 W 34TH ST 17TH FLOOR NEW YORK, NY 100012320 **DUNS:** 077090066 **EIN:** 1311726494A1 **RECIPIENT ID:**
Change of Contact PD/PI: N/A	
Administrative Official: ALEKSEI CHMURA 460 W 34th St., 17th Floor New York, NY 10001 **Phone number:** (b) (6) **Email:** (b) (6)	**Signing Official:** ALEKSEI CHMURA 460 W 34th St., 17th Floor New York, NY 10001 **Phone number:** (b) (6) **Email:** (b) (6)
Human Subjects: Yes HS Exempt: No Exemption Number: Phase III Clinical Trial:	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Zoonotic coronaviruses are a significant threat to global health, as demonstrated with the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, and the recent emergence Middle East Respiratory Syndrome (MERS-CoV). The wildlife reservoirs of SARS-CoV were identified by our group as bat species, and since then hundreds of novel bat-CoVs have been discovered (including >260 by our group). These, and other wildlife species, are hunted, traded, butchered and consumed across Asia, creating a largescale human-wildlife interface, and high risk of future emergence of novel CoVs.
To understand the risk of zoonotic CoV emergence, we propose to examine 1) the transmission dynamics of bat-CoVs across the human-wildlife interface, and 2) how this process is affected by CoV evolutionary potential, and how it might force CoV evolution. We will assess the nature and frequency of contact among animals and people in two critical human-animal interfaces: live animal markets in China and people who are highly exposed to bats in rural China. In the markets we hypothesize that viral emergence may be accelerated by heightened mixing of host species leading to viral evolution, and high potential for contact with humans. In this study, we propose three specific aims and will screen free ranging and captive bats in China for known and novel coronaviruses; screen people who have high occupational exposure to bats and other wildlife; and examine the genetics and receptor binding properties of novel bat-CoVs we have already identified and those we will discover. We will then use ecological and evolutionary analyses and predictive mathematical models to examine the risk of future bat-CoV spillover to humans. This work will follow 3 specific aims:

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces. We will examine if: 1) wildlife markets in China provide enhanced capacity for bat-CoVs to infect other hosts, either via evolutionary adaptation or recombination; 2) the import of animals from throughout Southeast Asia introduces a higher genetic diversity of mammalian CoVs in market systems compared to within intact ecosystems of China and Southeast Asia; We will interview people about the nature and frequency of contact with bats and other wildlife; collect blood samples from people highly exposed to wildlife; and collect a full range of clinical samples from bats and other mammals in the wild and in wetmarkets; and screen these for CoVs using serological and molecular assays.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk. We propose two competing hypotheses: 1) CoV host-range in bats and other mammals is limited by the
phylogenetic relatedness of bats and evolutionary conservation of CoV receptors; 2) CoV host-range is limited by geographic and ecological opportunity for contact between species so that the wildlife trade disrupts the ‘natural’ co-phylogeny, facilitates spillover and promotes viral evolution. We will develop CoV phylogenies from sequence data collected previously by our group, and in the proposed study, as well as from Genbank. We will examine co-evolutionary congruence of bat-CoVs and their hosts using both functional (receptor) and neutral genes. We will predict host-range in unsampled species using a generalizable model of host and viral ecological and phylogenetic traits to explain patterns of viral sharing between species. We will test for positive selection in market vs. wild-sampled viruses, and use data to parameterize mathematical models that predict CoV evolutionary and transmission dynamics. We will then examine scenarios of how CoVs with different transmissibility would likely emerge in wildlife markets.

Specific Aim 3: Testing predictions of CoV inter-species transmission. We will test our models of host range (i.e. emergence potential) experimentally using reverse genetics, pseudovirus and receptor binding assays, and virus infection experiments in cell culture and humanized mice. With bat-CoVs that we’ve isolated or sequenced, and using live virus or pseudovirus infection in cells of different origin or expressing different receptor molecules, we will assess potential for each isolated virus and those with receptor binding site sequence, to spill over. We will do this by sequencing the spike (or other receptor binding/fusion) protein genes from all our bat-CoVs, creating mutants to identify how significantly each would need to evolve to use ACE2, CD26/DPP4 (MERS-CoV receptor) or other potential CoV receptors. We will then use receptor-mutant pseudovirus binding assays, in vitro studies in bat, primate, human and other species’ cell lines, and with humanized mice where particularly interesting viruses are identified phylogenetically, or isolated. These tests will provide public health-relevant data, and also iteratively improve our predictive model to better target bat species and CoVs during our field studies to obtain bat-CoV strains of the greatest interest for understanding the mechanisms of cross-species transmission.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Year 2 NIAID CoV Report Final.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: Year 2 NIAID CoV Report Professional Development.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

1) Conference and University lectures: PI Daszak, and Co-investigators Shi, Epstein, Olival, Ge, and Zhang gave >100 invited University and Conference lectures including Forum on Microbial Threats (National Academies of Science), Symposium at École du Val-de-Grâce in Paris, Leadership Roundtable at Concordia University Montreal, 1st annual Global Pandemic Policy Summit at Texas A&M Univ., Intl. Conf. of the Wildlife Disease Association in Australia, Intl. Conf. of Conservation Biol in Montpellier France, Michigan State University, Duke University, WDA, ISID conference, Zoological Society of London Symposium, Future Earth meeting, North American Bat Research Symposium, and others that included specific discussion of the current project and results.

2) Agency and other briefings: PI Daszak and Research Technician Dr. Guangjian Zhu introduced this project to potential collaborators within the following agencies: Forestry Dept of Peoples' Republic of China, FAO, TNC, TRAFFIC, China CDC, and TA Foundation in Beijing China in meetings (2015) and also at presentations at the first Wildlife and Public Health Workshop in China (2016) co-hosted by EcoHealth Alliance, the State Forestry Administration of China, and China CDC.

3) Public outreach: PI Daszak presented this work to members of the NIH, NSF, DoD, IUCN, EPA, and the general public, at an EcoHealth Alliance meeting hosted by the Cosmos Club, Washington D.C. (2015); PI Daszak and Co-investigator Zhu reported on this project at a Wildlife Trade and Public Health Seminar, Beijing (2016); PI Daszak introduced this project in a lecture on Pandemics at a New York Academy of Science Panel (2016); Co-PI Y-Z Zhang presented project and results-to-date to department heads and senior researchers at Infectious Disease Departments of four Yunnan Hospitals (2015)

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces.

- Given the reduced amount of wildlife in the local markets within Southern China, and the continued expansion of the Chinese wildlife trade within SE Asia, we would like to conduct short field trips to assess markets, identify wildlife in them, and sample species of bats and other high-risk hosts in countries that neighbor China (Myanmar, Vietnam, Cambodia, Lao PDR) and others that supply wildlife to the international trade to China (Thailand, Malaysia, Indonesia. EcoHealth Alliance has other activities in these countries which would provide leverage to reduce costs of fieldwork, and samples would be tested in Wuhan, China.

- Following the successful collection of ethnographic interviews and focus groups in Year 2, we will be analyzing the qualitative data collection from Years 1 and 2.

- Finalize and conduct survey collection tool for a network study of wildlife farmers using a questionnaire to characterize and map the wildlife value chain.

- After the success of our pilot studies in Year 2, we will continue targeted (at individuals with high risk of exposure to bats), integrated behavioral and biological survey work in Yunnan and expand to Guangxi and Guangdong provinces.

- We will commence our anonymized, surveillance data collection from acutely ill hospital in-patients who satisfy syndromic eligibility criteria; have complete medical records; non-normative laboratory confirmed diagnostic results; and suspected acute viral infection. Eligibility criteria are: (a) suspected acute viral infection; (b) fever > 38˚C, and (c) presenting symptoms of at least one of the following:
•Encephalitis of unknown origin
•Hemorrhagic fever of unknown origin
•Respiratory disease
oInfluenza-like illness (ILI)
oSevere Acute Respiratory like Illness (SARI)
•Rash
•Diarrhea
Some patients with particular infections such as with HIV, HCV, and HBV, may be excluded from the study on that basis. Hospital surveillance has the advantage of monitoring an acutely ill population. Anonymized, passive hospital surveillance allows for data collection and viral testing from all eligible hospital patients thereby limiting population sample bias and increasing the likelihood of identifying positive cases. The strengths of this approach are enormous: an unbiased patient population; prospectively collected, anonymized patient data; a low resource effort with a high efficiency design; and impactful research potential for both case series and case control studies. We have already secured approval from the Institutional Review Boards of the Wuhan School of Public Health and Hummingbird IRB.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk.
Future steps to optimize the model of role of species diversity in CoV emergence risk will include:

- Test and implement our respondent-driven survey to collect specific data on the diversity, abundance, and turnover of species along the wildlife trade network in south China.

- Model viral mixing across the full range parameters found along the wildlife trade network to identify the trade nodes with highest mixing potential. This will include a network analysis of market facility/site connectivity including wild harvest sites, wildlife farming operations, transit holding facilities, and small and large wildlife markets.

- Phylogeographic study of bat-CoV to better understand the geographic distribution and evolution of bat-CoV genetic diversity in south

China.

- Phylogeographic study of bat host (Rhinolophus) species to assess the connectivity of bat populations and infer their historical movements and demographic history to improve our understanding of CoV transmission among bat populations in southern China. Preliminary sequences data has been generated and will be completed and analyzed.

- Cophylogenetic analyses of bat host and CoV phylogenies to assess frequency of cross-species transmission. Comparison of Alpha- and Beta-CoV cophylogenetic patterns building on Year 2 analyses using published sequences and also including Spike gene and additional sequences obtained in Year 2.

- Test and implement our respondent-driven survey to assess diversity, abundance, and turnover of species along the wildlife trade network.

- Examine co-evolutionary congruence of bat-CoVs and their hosts using both functional (receptor) and neutral genes;

- Parameterize mathematical models that predict CoV evolutionary and transmission dynamics
- Continued surveillances of SARS-like CoVs and lineage C betacoronaviruses (MERS-related CoVs) in Southern China;

- Full-length genome sequencing and evolution analysis of SARS-like coronaviruses identified from different bat species and different geographical locations across China;

- Full-length genome sequencing and evolution analysis of Lineage C betacoronaviruses identified from different bat species and different geographical locations across China;

- Full-length genome sequencing and evolution analysis of HKU9-related and HKU10-related bat coronaviruses in China;

Specific Aim 3: Testing predictions of CoV inter-species transmission. The following experiments will be undertaken in Year 2:

- Humanized mice with human ACE2 receptors will be infected with WIV1 and the two rescued chimeric SARS-like coronaviruses to determine the tissue tropism and pathogenicity of bat SL-CoV

- Isolation of novel bat coronaviruses. Live virus or pseudovirus will be used to infect cells of different origin or expressing different receptor molecules. Spillover potential for each isolated virus will be assessed.

- An infectious clone of full-length MERS-CoV will be constructed using reverse genetic method. Using the S sequence of different MERS-related viruses identified from Chinese bats, the chimeric viruses with S gene of bat MERS-related coronaviruses and backbone of the infectious clone of MERS-CoV will be constructed to study the receptor usage and infectivity of bat MERS-related coronavirus.

- Surveillance of infection in human populations by SARS-like CoVs. This work will be performed at locations in Yunnan, Guangxi, and Guangdong provinces, in previously identified areas with human populations of high risk of exposure to bats. PCR and ELISA will be used, respectively, for detection of viral replicase gene and antibodies against the viral nucleocapsid protein.

1R01AI110964 Year 2 Report PI: Daszak, Peter

Year 1 Report: Understanding the Risk of Bat Coronavirus Emergence

Award Number: 1R01AI110964-02

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Section B: Accomplishments

B.1 What are the Major Goals of the Project

Zoonotic coronaviruses are a significant threat to global health, as demonstrated with the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, and the recent emergence Middle East Respiratory Syndrome (MERS-CoV). The wildlife reservoirs of SARS-CoV were identified by our group as bat species, and since then hundreds of novel bat-CoVs have been discovered (including >260 by our group). These, and other wildlife species, are hunted, traded, butchered and consumed across Asia, creating a largescale human-wildlife interface, and high risk of future emergence of novel CoVs. To understand the risk of zoonotic CoV emergence, we propose to examine 1) the transmission dynamics of bat-CoVs across the human-wildlife interface, and 2) how this process is affected by CoV evolutionary potential, and how it might force CoV evolution. We will assess the nature and frequency of contact among animals and people in two critical human-animal interfaces: live animal markets in China and people who are highly exposed to bats in rural China. In the markets we hypothesize that viral emergence may be accelerated by heightened mixing of host species leading to viral evolution, and high potential for contact with humans. In this study, we propose three specific aims and will screen free ranging and captive bats in China for known and novel coronaviruses; screen people who have high occupational exposure to bats and other wildlife; and examine the genetics and receptor binding properties of novel bat-CoVs we have already identified and those we will discover. We will then use ecological and evolutionary analyses and predictive mathematical models to examine the risk of future bat-CoV spillover to humans. This work will follow 3 specific aims:

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces. We will examine if: 1) wildlife markets in China provide enhanced capacity for bat-CoVs to infect other hosts, either via evolutionary adaptation or recombination; 2) the import of animals from throughout Southeast Asia introduces a higher genetic diversity of mammalian CoVs in market systems compared to within intact ecosystems of China and Southeast Asia; We will interview people about the nature and frequency of contact with bats and other wildlife; collect blood samples from people highly exposed to wildlife; and collect a full range of clinical samples from bats and other mammals in the wild and in wetmarkets; and screen these for CoVs using serological and molecular assays.

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data to parameterize mathematical models that predict CoV evolutionary and transmission dynamics. We will then examine scenarios of how CoVs with different transmissibility would likely emerge in wildlife markets.

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B.1a Have the major goals changed since the initial competing award or previous report? No.

B.2 What was accomplished under these goals?

<u>Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces</u>

In year 2, we continued and expanded the qualitative research begun at the end of Year 1. In addition, a community based integrated biological behavioral surveillance system was developed and pilot tested to identify specific animal exposure risk factors associated with biological evidence of exposure to SARS-like CoV (i.e., seropositive status).

QUALITATIVE RESEARCH

Targeted, in-depth ethnographic interviews were conducted with 47 individuals (18 women; 29 men) in rural Southern China where wildlife trade routes have been documented. Yunnan, Guangxi and Guangdong provinces were specifically selected for study because they have large wildlife populations, a diversity of wildlife species and numerous live animal markets. Individuals who were 18 years of age or older and who were able to provide informed consent were eligible to participate. Twenty-three (49%) in-depth interviews were conducted in Yunnan province at nine different sites, 24 (51%) in Guangxi province at six different sites. In addition, one focus group was conducted in Guangxi. The study was approved by the Institutional Review Boards of the Wuhan School of Public Health and Hummingbird IRB.

Recruitment sites in each province included forested areas or preserves, wildlife farms, hunting areas, wildlife restaurants, live animal markets, caves where people dwell or collect guano and residential areas/farms near known bat caves or roosts. Participants were recruited primarily through local contacts developed as part of wildlife conservation and health research conducted by team members over the past decade. Contacts including wildlife conservationists and researchers, local government health outreach workers and wildlife farmers facilitated introductions and provided referrals. To achieve a sample with sufficient representation of categories of interest, participants were recruited using

purposive sampling, which provides minimum quotas in terms of sex, age and wildlife exposure setting (e.g., live animal market, forest preserve).

The five core themes that guided the in-depth discussions are: 1) human-animal contact, 2) unusual illness experience and response, 3) socioeconomics and daily living, 4) biosafety and 5) human environments and movement/travel. An ethnographic interview guide was developed with examples of questions that could be asked for each theme. In addition, field based participant-observation was ongoing throughout the study and involved observing and talking informally with people in their own natural setting. Field notes were maintained of these ongoing observations and discussions.

Table 1: Species Observed in Wetmarkets in Guangdong Province from 2015 - 2016

Genus species	Common Name
Prionailurus bengalensis	Leopard Cat
Nyctereutes procyonoides	Raccoon Dog
Sus scrofa	Wild Boar
Lepus sinensis	Chinese Hare
Arctonyx collaris	Hog Badger
Hystrix brachyura	Porcupine
Marmota sp.	Marmot
Rhizomes sinensis	Bamboo Rat
Erinaceus sp.	Hedgehog
Mustela putorius	Ferrets
Muridae	Rat (species unknown)
Myocastor coypus	Nutria
Vulpes sp.	Fox
Mustela sibirica	Siberian weasel
Paguma larvata	Masked Palm Civet
Felis catus	Domestic Cat
Canis lupus familiaris	Domestic Dog
Cervinae	Sambar Deer
Ovis aries	Sheep
Capra sp.	Domestic Goat
Ratus norvegicus	Common Rat

Interviews were conducted between March and June 2105 by 10 trained interviewers, none of whom had social science training. Interviewers conducted between one and 22 interviews; three interviewers conducted two thirds of all interviewers. Interviews lasted between 20 and 60 minutes, and were tape-recorded and transcribed verbatim before they were translated into English. All participants received cooking oil valued at US$10 in appreciation of their time.

The data are currently being coded and an analytic database is being constructed. Initial insights include observations by a number of participants, especially those who are older, that there has been a decrease in wildlife in the surrounding environment. This decrease is attributed to many factors including infrastructure development. The government has invested resources to build new roads and renovate local infrastructure with the intention of increasing tourism. This has reduced forested area.

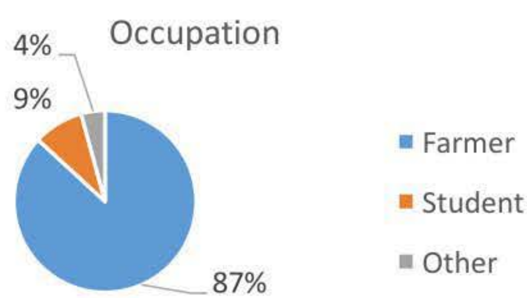
Observations by research staff in live animal markets in Guangzhou found wildlife to be plentiful (see Table 1), although no bats were seen for sale during the observation period. In contrast, wildlife was not found in live animal markets at the sites we visited in either Yunnan or Guangxi. This is a change from previous research visits to the same or similar communities, when bats, rodents and wild boar could be found. Locals in Yunnan and Guangxi attribute the change to conservation law enforcement. The success of conservation enforcement may have moved hunting and trapping underground and made the capture of local wildlife less economically feasible than other income generating activities.

Preliminary analyses are underway. Three specific studies in support of Specific Aim 1 are being developed: the changing wildlife trade in Southern China, the economics of wildlife farming, and zoonotic disease risks resulting from a rapidly changing wildlife trade.

INTEGRATED BIOLOGICAL BEHAVIORAL SURVEILLANCE PILOT STUDY

Currently, mechanisms of zoonotic viral spillover are unknown. In order to evaluate potential risk factors, it is necessary to measure both exposure and outcome data. Therefore, a behavioral risk survey was developed that assessed both animal exposure and experiences of unusual illness both during lifetime and in the past 12 months. In addition, participants were requested to provide serum to test for previous exposure to SARS-like CoV. The integrated surveillance was pilot tested in October 2015 among residents living near bat caves or roosts where SARS-like-CoV has been previously detected in the bat population in Jinning County, Yunnan. Please view the full survey here:

https://www.dropbox.com/s/sv62neywuvl027r/Questionnaire%20Complete.docx?dl=0



Of 218 participants, 139 (64%) were women and 79 (36%) were men, with a mean age of 48 (range: 12-80). Most reported being farmers (87%, and see chart to left); a majority were long term residents (97%). Animal exposures in the past year were extensive, including general (e.g., buying live animals at markets [61%]) and intimate (e.g., being scratched or bitten [9%], slaughter [38%]). In fact, two-thirds of participants reported handling recently killed animal parts and 2 out of 5 reported slaughtering animals. Only 20 (9%) participants reported known exposure to bats.

Standardized syndromic case definitions informed questions concerning unusual illness experience (e.g. severe acute respiratory infections [SARI], influenza-like illness [ILI]). Lifetime, 12 month and unusual illness experience in family for the past 12 months were assessed for all participants. In the past year, SARI was reported by 4 (2%) respondents and for 4 additional family members. Table 2 provides data for all unusual illness experience assessed. None of the participants were found to be seropositive for SARS-like CoV.

Table 2. Unusual Illness Experience

Symptoms	Ever	Past 12 months	Family (12m)
Severe Acute Respiratory Infections (SARI)	15 (6.9%)	4 (1.8%)	4 (1.8%)
Influenza Like Illness (ILI)	54 (24.8%)	16 (7.3%)	26 (11.9%)
Encephalitis	19 (8.7%	4 (1.8%)	3 (1.4%)
Hemorrhagic Fever	0 (0.0%)	0 (0.0%)	0 (0.0%)
Fever with Diarrhea /Vomiting	12 (5.5.%)	2 (0.9%)	3 (1.4%)
Fever with Rash	2 (0.9%)	2 (0.9%)	3 (1.4%)

Although the sample size was small, animal exposures among those who reported unusual illness experiences in the past 12 months were evaluated. Of the four respondents who reported SARI symptoms, 75% reported: raising animals, animals in the home, preparing recently killed animals and buying live animals; 50% reported slaughter. Among the 16 respondents who reported ILI symptoms, 12 (75%) reported handling/preparing recently killed animals, 11 (69%) Handling live animals or having animals in the home, 10 (63%) reported slaughtering/killing animals or buying live animals at wet market, 9 (56%) raised live animals, 7 (44%) reported a pet, and 1 (6%) reported animal feces near food or eating animal touched or damaged food, hunting, or eating raw/undercooked animal products. Finally, among the four respondents who reported encephalitis symptoms, 3 (75%) reported hunting, handling or raising animals, 2 (50%) reported animals in the home, 1 (25%) reported having animals as pets, slaughtering/killing animals, or having bought live animals at wet market.

Respondents were asked about the source of their unusual illnesses. None reported any kind of animal exposure as a potential source of infection and most stated they had no idea how they had become infected. However, when asked about potential behavior changes made at live animal markets in the last 12 months, participants reported a great deal of change. In particular, respondents reported buying live animals less often (38%), only buying farmed wildlife (54%) or buying meat at the supermarket (23%). (See Table 3).

Table 3: Behavior Change at Wet Market in the last 12 months

Behavior	N	(%)
Wear a mask	4	(3.0)
Wear gloves	5	(3.8)
Wash hands	80	(60.6)
Sometimes shop for meat at supermarket	30	(22.7)
Buy live animals less often	50	(37.9)
Buy only farmed wildlife	71	(53.8)
No longer buy wildlife at wet market	39	(29.5)

The results of this pilot study conducted with a largely female farmer population found high levels of unusual illness, as well as high levels of exposure to animals. There was a notable lack of knowledge of animals' ability to transmit infection. Despite this lack of knowledge, there may be a sense of unease about animal exposures, given the fairly dramatic behavior changes reported at live animal markets. The finding of a reduction in wildlife purchase may be due to sensitivity to the legality of wildlife trade, biasing respondents towards not admitting purchasing wildlife. Although, there were no participants seropositive for SARS-like CoV, serological data may add support to the findings from self-reported syndromic surveillance, once serological assays are optimized.

In preparation for full implementation of the integrated biological behavioral surveillance, the survey has been programmed as an application for use on either a mobile device or computer. Electronic data collection will facilitate survey implementation in the field and quality control of the data being collected. Four field team leads were trained on behavioral survey data collection, data collection technologies (the tablet application) and analysis.

Nucleic acid test results of human biological samples

Testing High-Risk Human Populations for Coronavirus Infection

Surveillance of CoV infections in human populations by SARS-like CoVs was significantly expanded in Year 2, including both custom-built ELISA serology (an assay developed by the Wuhan Institute of Virology to test antibodies against the N protein of SL-CoV) and PCR detection of viral RNA.

Serological test for SL-CoV antibodies in human samples from Jinning, Yunnan Province
In order to assess past exposure to bat CoVs, 223 human sera samples were collected in villages in proximity to the bat habitat from which two SL-CoVs with potential for interspecies infection, WIV1 and WIV16, were discovered in our previous research. An ELISA developed by the Wuhan Institute of Virology was used to test antibodies against the N protein of SL-CoV. A number of human specimens generated high OD values and neutralization test to WIV1 and WIV16 was then performed. These findings are encouraging; however, no neutralization antibodies were detected. In Year 3, we will continue to validate and optimize these ELISA assays and other serological tests to obtain data on past CoV exposure.

PCR test for CoV Nucleic Acid in human samples from several Provinces
We tested 405 individual human samples for CoV RNA to identify evidence of active infection in human populations and to obtain sequence data on strain variation. Individual samples (4 each) were pooled prior to nucleic acid extraction then tested using PCR. When a group tested positive, we then conducted the confirmation test in the individual samples. One single sample (14XN611) from someone who had identified as having had a fever and suffered both a cough and headache in the past 7-days was then identified to be positive for HCoV-HKU1. The low number of PCR detections in human specimens is not unexpected, and will be improved in Year 3-5 by better targeting syndromic individuals for specimen collection and continuing to optimize PCR assays. Refined serological assays (above) will provide sufficient data to assess past exposure to specific CoV lineages, and optimizing of PCR detections will allow for more CoV positive human sequences moving forward.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk

Bat CoV PCR detection and sequencing from live-sampled bat populations
We collected 1,714 anal swab samples, 677 fecal samples, 53 blood samples, and 38 serum samples from 15 bat genera in Guangdong, Yunnan, Sichuan, Hubei, Hunan, Guizhou, Guangxi provinces (Table 4).

Table 4 Bat Samples collected for CoV surveillance in 2015

Sample date	Sample location	Anal	Fecal	Blood	Serum
Mar. 2015	Huidong, Guangdong	69	--	--	--
Jun. 2015	Guangdong	495	--	12	--
Apr. 2015	Menglun, Yunnan	51	--	--	--
May 2015	Jinning, Yunnan	--	193	--	--
May. 2015	Mojiang, Yunnan	93	--	--	--
Oct. 2015	Jinning, Yunnan	30	--	--	--

Dec, 2015	Jingna, Yunnan	15	15	13	13
	Miaoxin, Yunnan		42	28	25
Jul, 2015	Zigong, Sichuan	128	--	--	--
Aug, 2015	Hubei		332		
Sep, 2015	Xianning, Hubei		95		
Aug, 2015	Jishou, Hunnan	204			
Aug-Sep, 2015	Tongren, Guizhou	438			
Dec, 2015	Longzhou, Guangxi	191			
	Total	1714	677	53	38

We tested 2,256 samples for CoV RNA and 280 tested positive. The total positive rate is 12.4% (Table 5). Diverse alphacoronaviruses related to Bat CoV 1A, 1B, HKU2, HKU6, HKU7, HKU8 and HKU10 were identified; SARS-like coronaviruses were detected in *Rhinolophus* bats in both Yunnan and Guangdong (Fig 1). Novel lineage B betacoronaviruses more distantly related to SARS-CoV than other SL-CoVs were detected in *Vespertilo superans* in Sichuan. HKU4-related coronaviruses were found in *Tynolycteris pachypus* in Guangdong and Guangxi while HKU5-related coronaviruses were found to be highly prevalent in *Vespertilio superans* in Zigong, Sichuan (41 bats out of 128 tested positive).

Table 5 Test result of bat CoV surveillance in 2015 – 12% positive (280/2,256)

	Yunnan	Guangdong	Hubei	Sichuan	Guangxi	Guizhou	Hunan	Total
Bat species	**No.positive/No.tested**							
Rhinolophus spp.	47/98	12/103				16/225	8/63	83/489
Hipposideros spp.	0/35	0/51	26/152			0/131	0/91	26/460
Ia io						0/3		0/3
Pipistrellus spp.	1/1	0/19				0/2	0/4	1/26
Miniopterus spp.	6/7	34/83				2/6		42/96
Eonycteris spp.	0/3							0/3
Vespertilio superans				41/128				41/128
Myotis spp.		1/38				0/70	0/35	1/143
Taphozous spp.	0/25					0/1		0/26
Tynolycteris pachypus		8/25			27/191			35/216
Scotophilus kuhlii		1/1						1/1
Eptesicus fuscus		0/1						0/1
Tadrida spp.		0/5						0/5
Barbastella							0/1	0/1
Nyclatus velutiaus							0/10	0/10
Fecal samples	28/468		22/180					50/648
Sub-total	82/637	56/326	48/332	41/128	27/191	18/438	8/204	**280/2256**

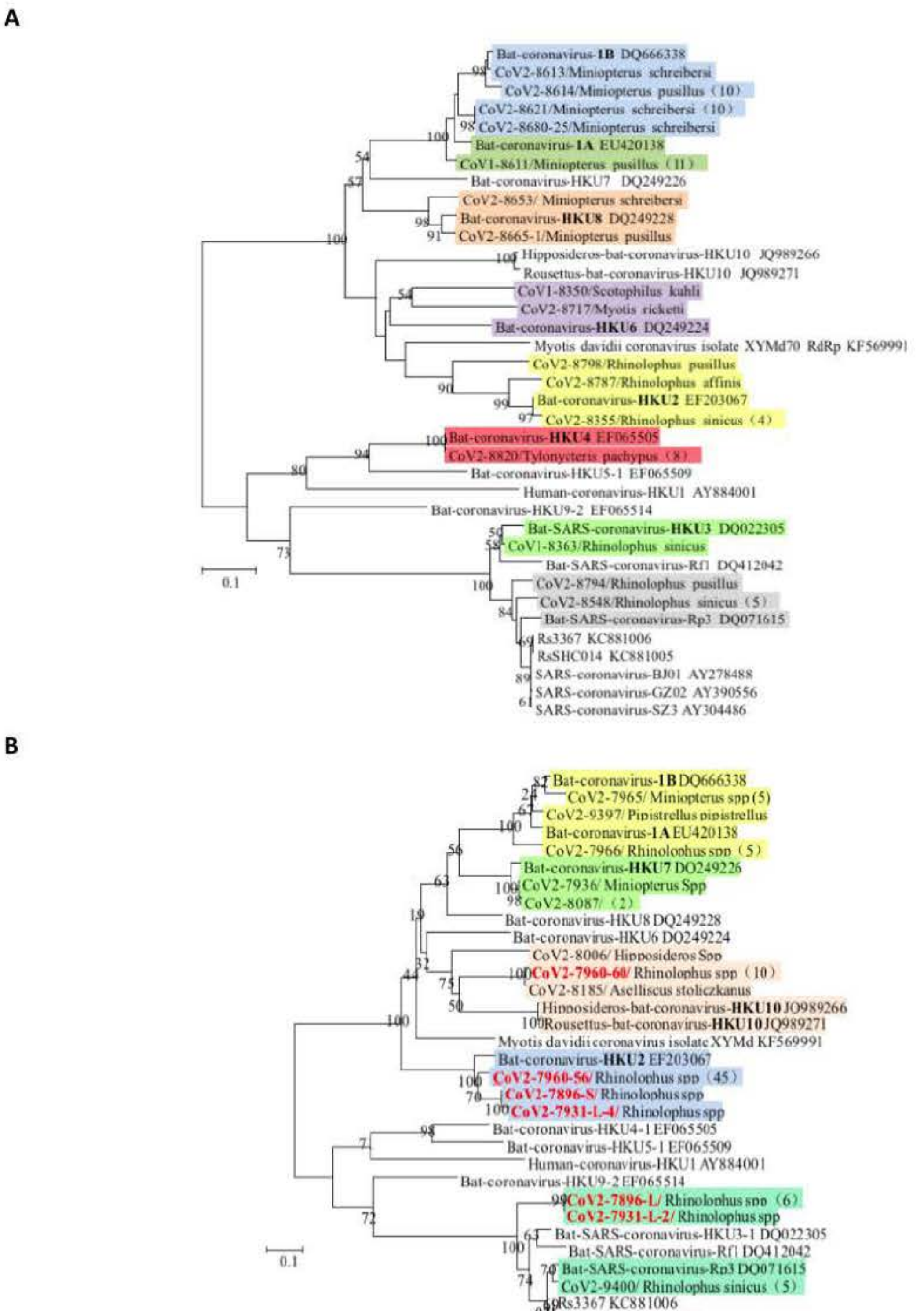


Fig 1 Phylogenetic analysis of partial RdRp gene of CoV (440-nt partial sequence). CoVs identified in 2015 are named by the sample numbers. Sequence amplified from samples co-infected with two CoV strains are indicated in red. (A) CoVs detected in Guangdong. (B) CoVs detected in Yunnan.

Cophylogenetic analysis of CoV host switching

We completed preliminary cophylogenetic analysis of bat host – CoV sequences using data published in the literature and available on Genbank. Two figures from these analyses are highlighted below (Figs 2 and 3) and these methods are currently being extended using partial RdRp CoV and bat mitochondrial DNA sequences from a large number of bat specimens found CoV positive in Year 2 (Table 5, above).

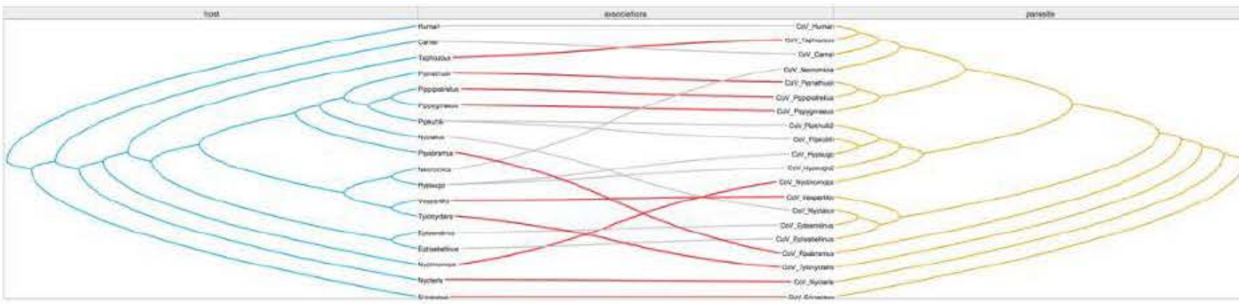


Figure 2: Tanglegram depicting the pattern of infection of bats (and outlier mammalian hosts) by CoVs. The CoV tree was reconstructed from DNA sequences available in GenBank (partial RdRp gene) using Bayesian inference (MrBayes). The topology of host tree was reconstructed using the mammal and bat phylogenies available in Asher & Helgen (2010) and Agnarsson et al. (2011), using methods our group has previously applied to bat parasite cophylogenetic analyses (Lei and Olival 2014). Both ParaFit (ParaFitGlobal = 64957.61, p-value = 0.001) and PACo (m2 = 366.44, p-value = 0.013) provided evidence for significant global congruence between the two topologies, and evidence for coevolution. Lines connecting taxa indicate host-CoV associations. Red lines indicate significant host-CoV associations as indicated by ParaFit ($p \leqslant 0.05$, 999 permutations).

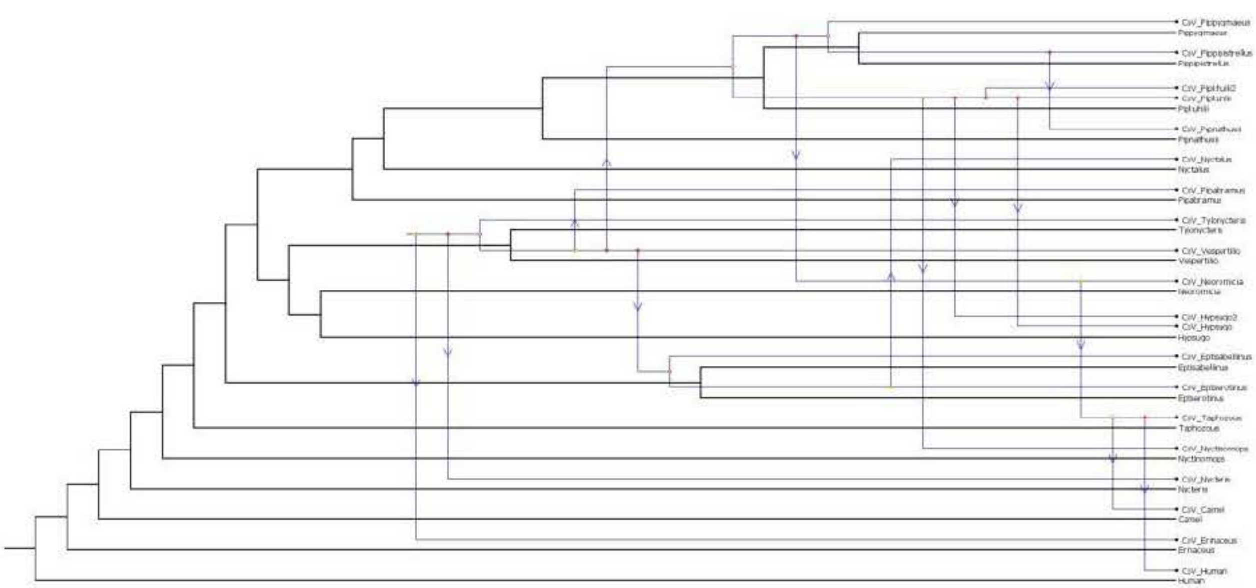


Figure 3: Reconstruction of one of 3 potentially optimal solutions of reconciled host-CoV trees recovered from a Jane analysis. Black and blue lines represent the host and CoV trees, respectively. For each solution, the number of co-speciation events inferred by Jane was always significantly greater than expected by chance. Jane inferred 4 co-speciation events (hollow colored circles), 1 duplication (solid

colored circle), 14 host switches (solid colored circle with arrow), 0 loss and 0 failure to diverge.

Our findings demonstrate co-speciation alone is not sufficient to explain the observed co-phylogenetic pattern and several host switches can be specifically identified. This is the case even if a significant global signal of co-speciation has been detected. This work highlights, the need for these types of detailed cophylgoenetic analyses to best explain the evolutionary history and host-switching of bat-CoVs.

References cited for the above analysis: Agnarsson, I., Zambrana-Torrelio, C.M., Flores-Saldana, N.P. & May-Collado, L.J. (2011) A time-calibrated species-level phylogeny of bats (Chiroptera, Mammalia). *PLOS Currents*, 3:RRN1212. Asher, R.J. & Helgen, K.M. (2010) Nomenclature and placental mammal phylogeny. *BMC Evolutionary Biology*, 10, 1-9. Lei BR, Olival KJ (2014) Contrasting Patterns in Mammal–Bacteria Coevolution: *Bartonella* and *Leptospira* in Bats and Rodents. *PLoS Negl Trop Dis* 8(3): e2738.

Market Characterization Model Parameterization

Our ongoing observational research and mapping of farms and markets suggests that rapid changes in the market and regulatory environment are changing the nature and location of the wildlife market trade. The nexus of the wildlife trade and the potential hotspots of interspecies viral mixing is now in many cases in animal storage facilities and transport between high-volume customers. To define realistic parameters for intermixing wildlife species in areas of high potential mixing, we have developed a preliminary survey and sampling protocol to assess these values as animals move along the value chain – through these storage facilities - using respondent-driven questionnaires to follow and sample along the wildlife trade network and reveal hidden nodes and sites of intermixing of species.

We have expanded our intermixing modeling framework to incorporate the variations along this value chain, where the diversity, abundance, residence time, and contact rates between species change as animals move through the trade network.

Specific Aim 3: Testing predictions of CoV inter-species transmission.

In Year 2, we continued surveillance for novel SARS-like CoVs from bats in Yunnan and Guangdong provinces and obtained full genome sequence for 11 CoV isolates. Full genome analysis of these CoV isolates was completed, including phylogenetic and recombination analyses. Importantly, recombination analysis of the full-length SL-CoV genome sequences from a single bat population revealed that frequent recombination events among different SL-CoV strains occur. Several SL-CoVs that are more genetically similar to SARS-CoV (2003) than any previously discovered were also identified from bat populations in Yunnan province. Full genome analysis suggests that an epicenter of SL-CoV occurs in rhinolophid bats and provides more insight into the evolutionary origin of SARS-CoV.

Full-length genome sequencing of SL-CoVs identified from a single bat colony

To date, including preliminary data submitted for this R01 that we are now analyzing under the current funding, we have conducted 5-years of surveillance of SL-CoV in a single bat colony in Yunnan Province (from 2011 to 2015), leading to the discovery of diverse novel SL-CoVs. Based on genotyping of these SL-CoVs by the region corresponding to the receptor-binding domain (RBD) of SARS-CoVs, 11 isolates were selected and full-length genome sequencing was performed in Year 2.

These SL-CoVs, including four others isolated previously from this colony, Rs3367, RsSHC014, WIV1 and WIV16, are highly diversified in the S gene, but share similar sequence identity to SARS-CoV in ORF1ab (Fig 4). Genomic phylogenetic analysis showed that the SL-CoVs detected in this colony are more closely

related to SARS-CoVs from other geographic regions, especially three isolates, WIV16, Rs4874 and Rs4231 (Fig 5). Notably, among the 15 SL-CoVs, two isolates, Rs4084 from *Rhinolophus sinicus* and Rf4092 from *Rhinolophus ferrumequinum*, are highly similar to SARS-CoV in the ORF8 region (Fig 5). Rf4092 possessed a single ORF8 of the same length (369bp) as that in civet SARS-CoV SZ3, and the sequence showed only 10 nucleotide substitution (Fig 6). The ORF8 sequence of Rs4084 is highly similar to that of Rf4092, however in the region corresponding to the 29-bp deletion acquired in human SARS CoVs (e.g Tor2), a shorter deletion of only 5-bp is present, resulting in two overlapping ORF8s, ORF8a and ORF8b. The position of start codon and stop codon of the two ORFs were consistent with those in human strains (Fig 6).

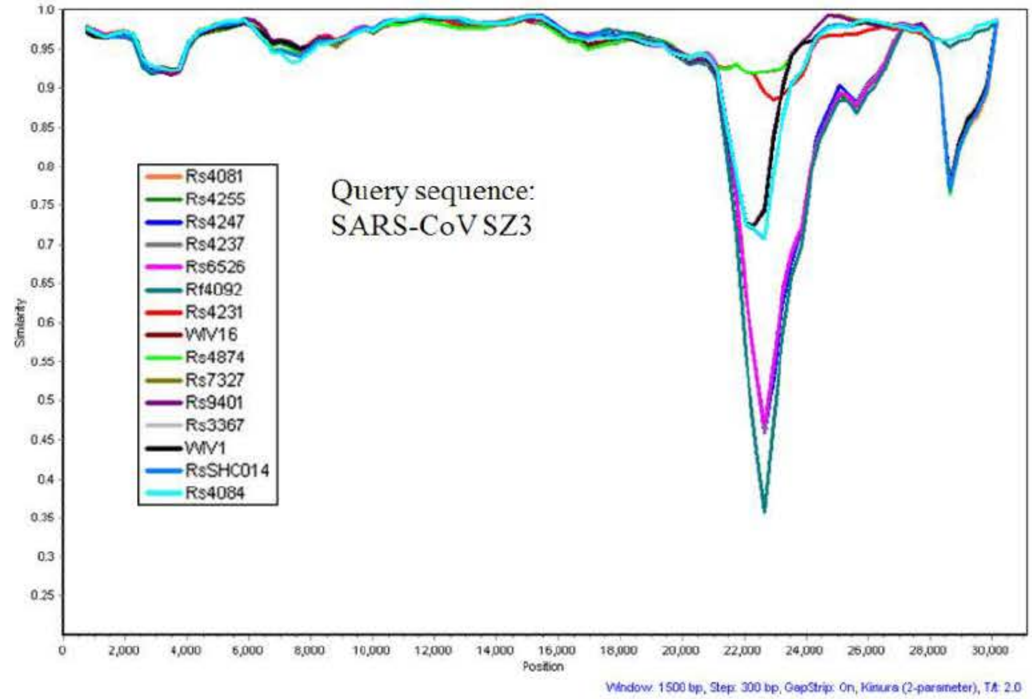


Fig 4. Simplot analysis of the 15 SL-CoVs identified from a single bat colony in Yunnan. SARS-CoV SZ3 is used as query sequence.

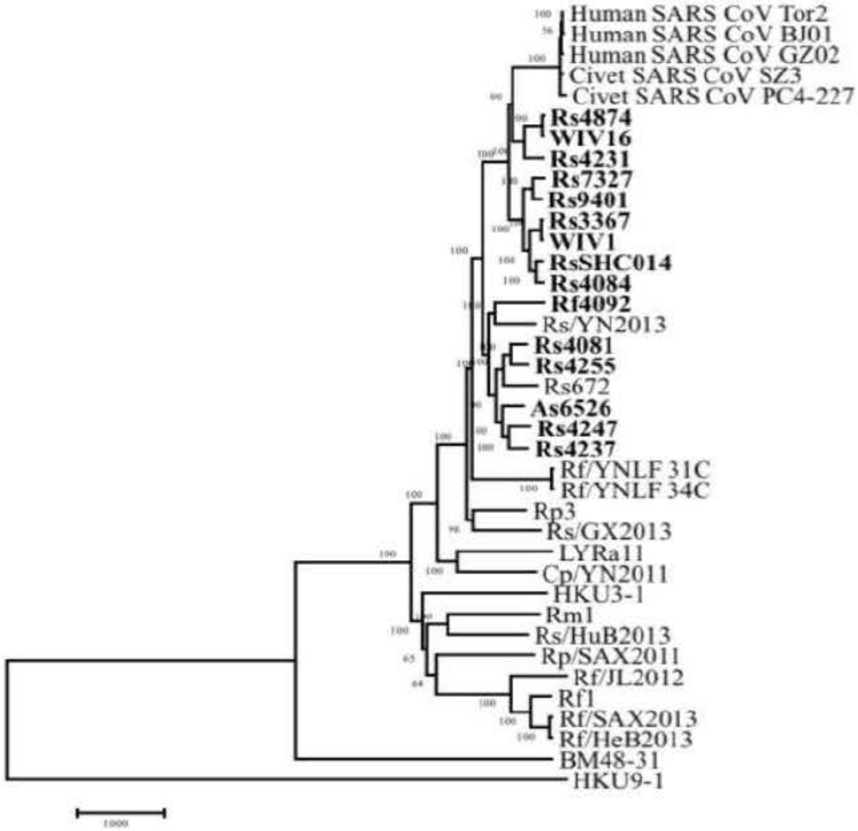


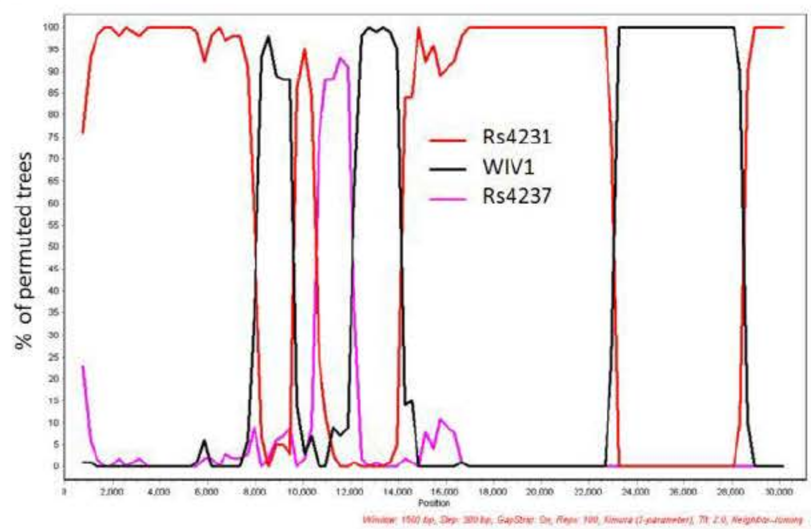
Fig 5. Phylogenetic analysis of full-length genome sequences of SL-CoVs and SARS-CoVs. Isolates identified in the single investigated bat colony in Yunnan in in bold.



Fig 6. Alignment of ORF8 nucleotide sequences of SARS-CoV and bat SL-CoVs. The red box indicates the 29-nt deletion present in SARS-CoV of middle and late phase.

Recombination analysis of the full-length genome sequences reveals frequent recombination events among different SL-CoV strains circulating in this bat population. For example, WIV16 appears to be a recombination product of WIV1 and Rs4231. An important breakpoint is identified between the N-terminal domain (NTD) and RBD region in the S gene (Fig 7A). Consequently, WIV16 is identical to Rs4231 and WIV1 in NTD and RBD of the spike protein, respectively, and is highly homologous to SARS-CoV in both NTD and RBD. This makes it the SL-CoV most closely related to the direct progenitor of SARS-CoV discovered to date. Moreover, evidence is found to support the hypothesis that the direct progenitor of SARS-CoV was generated from recombination of WIV16 with Rf4092 at the site near ORF8. This work, which identifies diverse SL-CoVs highly homologous to SARS-CoV in different regions of the genome, suggests that rhinolophid bats are an evolutionary epicenter of SL-CoV and offers more insights into the evolutionary origin of SARS-CoV.

A.



B.

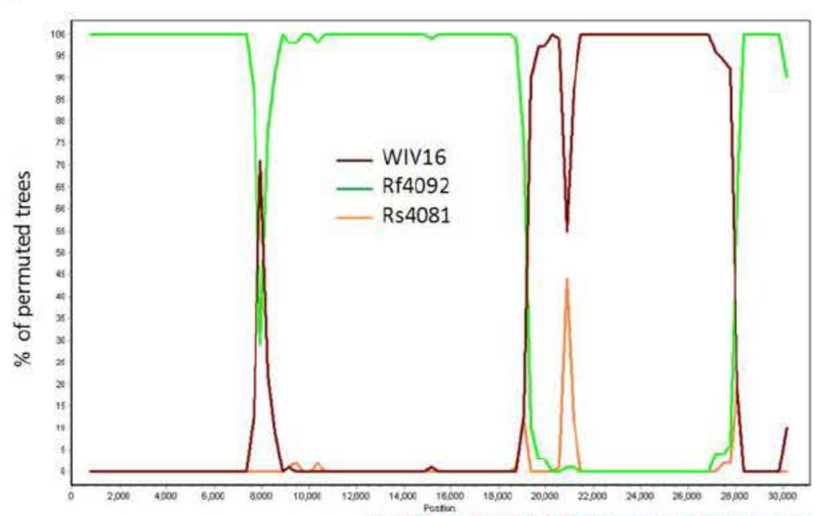


Fig 7 Bootscan analysis of full-length genome sequences of SL-CoVs. (A) WIV16 is used as query sequence. (B) SARS-CoV SZ3 is used as the query sequence. (Kimura model, window size, 1500bp, step size, 300bp)

Additional Year 2 items for Specific Aim 3:

- The infectious clone of WIV1 was successfully constructed using reverse genetic methods;
- Two chimeric bat SARS-like coronavirus strains were constructed by replacing the S gene in the backbone of WIV1;
- Permission to import mice with human ACE2 to China was obtained, so as to conduct the experimental infections proposed in our R01 specific aims.

Specific Goals Not Met.

- Comparative cophylogenetic analyses of bat host and CoV RdRp and Spike gene phylogenies, to assess patterns of evolutionary congruence and frequency of cross-species transmission (This will be conducted in year 3);
- Animal infection experiments of SARS-like coronaviruses were not done, because of the unavailability of mice with human ACE2 in Year 2. We now have secured these mice and will begin this work in year 3.
- Sampling of bat and other mammalian species in markets to screen for CoVs. We will begin this work in year 3.

Section C: Accomplishments: Publications

PUBLISHED

Xing-Yi Ge, Ning Wang, Wei Zhang, Ben Hu, Bei Li, Yun-Zhi Zhang, Ji-Hua Zhou, Chu-Ming Luo, Xing-Lou Yang, Li-Jun Wu, Bo Wang, Yun Zhang, Zong-Xiao Li, and Zheng-Li Shi. Coexistence of multiple coronaviruses in several bat colonies in an abandoned mineshaft. *Virologica Sinica* 31, 31–40 (2016).

Mei-Niang Wang, Wei Zhang, Yu-Tao Gao, Ben Hu, Xing-Yi Ge, Xing-Lou Yang, Yun-Zhi Zhang, Zheng-Li Shi. Longitudinal surveillance of SARS-like coronaviruses in bats by quantitative real-time PCR, *Virologica Sinica* 31(1): 78-80 (2016).

Cristin C. W. Young and Kevin J. Olival. Optimizing Viral Discovery in Bats. *PLoS ONE* 11(2) (2016).

Kevin J. Olival. To Cull, or Not To Cull, Bat is the Question. *Ecohealth* 13, 6–8 (2015).

Xing-Lou Yang, Ben Hu, Bo Wang, Mei-Niang Wang, Qian Zhang, Wei Zhang, Li-Jun Wu, Xing-Yi Ge, Yun-Zhi Zhang, Peter Daszak, Lin-Fa Wang, Zheng-Li Shi. Isolation and characterization of a novel bat coronavirus closely related to the direct progenitor of Severe Acute Respiratory Syndrome Coronavirus, *Journal of Virology* 90(6): 3253-6 (2015).

Ben Hu, Xingyi Ge, Lin-Fa Wang, Zhengli Shi. Bat origin of human coronaviruses. *Virology Journal* 12 (1): 221 (2015)

ACCEPTED, IN PRESS

Lei-Ping Zeng, Yu-Tao Gao, Xying-Yi Ge, Qian Zhang, Cheng Peng, Xinglou Yang, Bin Tan, Jing Chen, Aleksei Chmura, Peter Daszak, and Zheng-Li Shi. Bat SARS-like coronavirus WIV1 encodes an extra accessory protein ORFX involving in modulation of host immune response. *Journal of Virology* (in press, 2016)

B.4 What opportunities for training and professional development has the project provided?

We presented our project to graduate students, laboratory personnel, directors, and doctors from three Hospitals in Yunnan Province: Yunnan Provincial Institute of Endemic Diseases Control & Prevention (YNCDC); Dali Provincial Hospital; and The Third People's Hospital of Kunming. Select doctors at YNCDC (1) and Dali Provincial Hospital (3) were trained in the passive Hospital surveillance project protocols.

We trained graduate students from Dali School of Public Health (1) and the Wuhan University School of Public Health (3) in qualitative behavioral risk data collection methodologies and data collection technologies, survey data collection and analysis. These were also enrolled in and passed the Human Subjects Research Course provided by the Collaborative Institutional Training Initiative (CITI Program) at the University of Miami (http://citiprogram.org). The CITI Program is a leading provider of research education content with web based training materials serving millions of learners at academic institutions, government agencies, and commercial organizations in the U.S. and around the world.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Yang XL, Hu B, Wang B, Wang MN, Zhang Q, Zhang W, Wu LJ, Ge XY, Zhang YZ, Daszak P, Wang LF, Shi ZL. Isolation and Characterization of a Novel Bat Coronavirus Closely Related to the Direct Progenitor of Severe Acute Respiratory Syndrome Coronavirus. J Virol. 2015 Dec 30;90(6):3253-6. PubMed PMID: 26719272; PubMed Central PMCID: PMC4810638.
Complete	Olival KJ. To Cull, or Not To Cull, Bat is the Question. Ecohealth. 2016 Mar;13(1):6-8. PubMed PMID: 26631385; PubMed Central PMCID: PMC4833651.

Non-compliant Publications Previously Reported for this Project

Public Access Compliance	Citation
Non-Compliant	[redacted] (b) (4)

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
(b) (6) [redacted]	Y	DASZAK, PETER	(b) (6) [redacted]	(b) (6) [redacted]	BS,PHD	PD/PI	[redacted]	[redacted]	(b) (4), (b) (6) [redacted]			NA
	N	HOSSEINI, PARVIEZ RANA	(b) (6) [redacted]	(b) (6) [redacted]	BS,PHD	Co-Investigator	[redacted]	[redacted]	[redacted]			NA
(b) (6) [redacted]	Y	Ross, Noam Martin		(b) (6) [redacted]	PhD	Co-Investigator	[redacted]	[redacted]	[redacted]			NA
	N	OLIVAL, KEVIN J	(b) (6) [redacted]	(b) (6) [redacted]	PHD	Co-Investigator	[redacted]	[redacted]	[redacted]			NA
	N	KE, CHANGWEN			PHD	Co-Investigator	[redacted]	[redacted]	[redacted]	Center for Disease Control and Prevention of Guangdong Province	CHINA	NA
	N	ZHANG, SHUYI		(b) (6) [redacted]	PHD	Co-Investigator	[redacted]	[redacted]	[redacted]	East China Normal University	CHINA	NA
	N	ZHANG, YUNZHI		(b) (6) [redacted]	PHD	Co-Investigator	[redacted]	[redacted]	[redacted]	Yunnan Provincial Institute of Endemic Diseases Control & Prevention	CHINA	NA
	N	ZHU, GUANGJIAN		(b) (6) [redacted]	PHD	Co-Investigator	[redacted]	[redacted]	[redacted]	East China Normal University	CHINA	NA
	N	GE, XINGYI			PHD	Co-Investigator	[redacted]	[redacted]	[redacted]	Wuhan Institute of Virology	CHINA	NA
	N	EPSTEIN, JONATHAN H	(b) (6) [redacted]	(b) (6) [redacted]	MPH,DVM,BA,PHD	Co-Investigator	[redacted]	[redacted]	[redacted]			NA
	N	CHMURA, ALEKSEI A	(b) (6) [redacted]	(b) (6) [redacted]	BS	Non-Student Research Assistant	[redacted]	[redacted]	[redacted]			NA
	N	SHI,		(b) (6) [redacted]	PhD	Co-	[redacted]	[redacted]	[redacted]	Wuhan	CHINA	NA

		ZHENGLI				Investigator				Institute of Virology		

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

Yes

File uploaded: Noam Ross CV 2016.pdf

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

Noam Ross

(b) (6) [redacted]
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@noamross

EDUCATION

University of California — Davis, CA
Doctoral Candidate in Ecology — Expected Completion Summer 2015

- Dissertation Committee: Alan Hastings (major professor, Ecology), David Rizzo (Plant Pathology), Jim Sanchirico (Natural Resource Economics)
- Dissertation Research: "Managing Emerging Forest Disease Under Uncertainty"

Brown University — Providence, RI
Bachelor of Science in Environmental Science, Magna Cum Laude — May 2006

- Honors Thesis: "Soil Organic Matter in Northern Mongolia: Permafrost and Land-Use interactions"
- Phi Beta Kappa, Sigma Xi, Environmental Science Honors, Rosenberger Prize for Outstanding Service

SCIENTIFIC PUBLICATIONS

- Carl Boettiger*, **Noam Ross***, Alan Hastings (2013) *Early Warning Signals: The Charted And Uncharted Territories.* Theoretical Ecology http://dx.doi.org/10.1007/s12080-013-0192-6
- Fuller, Kate, David Kling, Kaelin Kroetz, **Noam Ross**, and James N. Sanchirico (2013) *Economics and Ecology of Open-Access Fisheries.* In: Shogren, J.F., (ed.) Encyclopedia of Energy, Natural Resource, and Environmental Economics, Vol. 2 Encyclopedia of Energy, Natural Resource, and Environmental Economics p.39-49. Amsterdam: Elsevier. http://dx.doi.org/10.1016/B978-0-12-375067-9.00114-5

In preparation

(b) (4) [redacted]

**Co-equal authorship*

POSTERS

- **Ross, Noam**. "Optimal Control of Disease in Space: An Approach Using Individual-based Models," June 1-4, 2014. 12th Annual Conference of Ecology and Evolution of Infectious Disease, Fort Collins, Colorado.
- **Ross, Noam**. "Designing Protective Treatments for Forest Disease Using a Spatial Point Process Model," November 20-21, 2014. California Forest Pest Council Annual Meeting, McClellan, CA.
- **Ross, Noam**. "Optimal Control of Forest Disease Under Changing Community and Spatial Structure," November 4-18, 2013. Sustainable Management of Natural Resources Workshop, Mathematical Biosciences Institute, Columbus, OH.

PRESENTATIONS

- **Ross, Noam**, "Fungal Disease Mortality: Modeling for Management of Sudden Oak Death." Dec 1, 2014 Invited talk at EcoHealth Alliance, New York, NY.
- **Ross, Noam**, "Modeling forest disease using a macroparasite framework ," Agust 13, 2014. 99th Annual Ecological Society of America Meeting, Sacramento, CA.
- Ashander, Jamie, Kelly Gravuer, Megan Kelso, Mary E. Mendoza and **Noam Ross** "Managing River-Floodplains Systems: A Historical and Ecological Perspective" September 14, 2002. Presentation at NSF REACH IGERT Floodplains Workshop

AWARDS + FELLOWSHIPS (*Total received $225,429*)

- Don Dahlsten Memorial Grant ($325) — California Forest Pest Council, 2012
  Designing Protective Treatments for Forest Disease Using Spatial Point Process Models
- NSF IGERT Bridge Fellowship ($57,500) — UC Davis, CA, 2012
  Managing Emerging Forest Disease Under Uncertainty
- NSF IGERT Traineeship in Rapid Environmental Change ($115,00) — UC Davis, CA, 2010
  Modifying River-Floodplain Systems: A Historical and Ecological Approach
- UC Davis Graduate Group in Ecology Fellowship ($40,604) — UC Davis, CA, 2010
- NSF Research Experience for Undergraduates Fellowship ($8,000) — Acad. of Natural Sciences, PA, 2005
- Undergraduate Research Fellowship ($4,000) — Brown University, RI, 2003

SERVICE + PROJECTS

- **Workshop Instructor,** Software Capentry and Data Carpentry Foundations — Jan 2015–Present
- **Student Rep,** UC Davis Graduate Group in Ecology Executive Committee — Sep 2013–Present
- **Reviewer:** *Theoretical Ecology* (4 reviews) — Feb 2013–Present
- **Web Developer and Technology Chair,** Ecology Graduate Student Association — June 2013–Present
  Creator + Maintainer of graduate student blog, resources, and news site (egsa.ucdavis.edu)
- **Founder + Organizer,** Davis R Users' Group — Sep 2012–Present
  Created users group that provides tutoring and seminars to graduate students in 10+ departments
- **Contributor,** R packages `knitr, knitcitations, rcrossref, rethinking` — 2012-Present
- **Organizer:** NSF REACH IGERT Workshop on Multiple Goals in Floodplain Restoration — Sep 2012
- **Organizer,** UC Davis Conference on Ecology and the Business Sector — Apr 2011
- **Organizer**, UC Davis Graduate Group in Ecology Symposium — May 2010-2011
- **External Reviewer,** World Resources Institute Corporate Ecosystem Services Review — Jan 2008
- **External Reviewer,** McKinsey-Clinton Global Initiative Forestry Project — Mar 2008
- **Business Stewardship Volunteer,** NY Coastal Marine Resources Center — Feb-Apr 2007

OTHER WORK EXPERIENCE

GreenOrder — New York, NY
Analyst, Senior Analyst: Corporate Environmental Strategy + Governance — Sep 2006–Oct 2009

- Conducted environmental performance analysis for products in energy, transportation, and water sectors
- Created green product metrics system R&D stage-gating system for construction products manufacturer
- Managed engagement with equipment rental company to identify growth opportunities in green building
- Performed market and competitive analyses for a wide array of clients in retail, real estate financial and cleantech sectors; prepared and delivered client presentations; managed projects
- Managed analysts performing environmental product certifications and market research
- Developed firm seminar series and analyst training materials; conducted trainings on topics including auditing, statistical analysis, and environmental performance benchmarking
- Audited certifications for environmental products and facility performance

Wal-Mart — Providence, RI
Contract Researcher/Consultant: Energy Efficient Products Initiative — May-Sep 2006

- Developed forecasting model for sales of energy-efficient lamps at Wal-Mart stores
- Created guidelines for design of lamp recycling program

Brown University Facilities Management Providence, RI
Administrative, Research, + Teaching Assistant: Energy and Design Jan 2003–May 2006

- Developed energy-use and financial projections for university energy usage scenarios
- Performed background research and feasibility analysis for university energy efficiency projects
- Provided tutoring, logistical support and web design for two courses in sustainable design
- Responsible for maintenance of energy efficient, low-impact building

Hovsgol Lake Global Environmental Facility and Brown University Mongolia + Providence, RI
National Science Foundation REU Fellow, Thesis Research June 2005-May 2006
Advisor: Clyde Goulden

- Independent research on climate-land use interactions on permafrost soil carbon storage
  Plant surveys, soil pit excavation, soil physical and chemical analysis, soil microbial process incubations

Marine Biological Laboratory Ecosystems Center Woods Hole, MA
Semester in Environmental Science Student Aug-Dec 2004
Advisor: Charles Hopkinson

- Examined effects of nitrogen pollution on structure of microplankton food webs
- Microcosm experiments, fluorescence microscopy, dissolved nutrient analysis, planktonic growth incubations

Brown Center for Environmental Studies Providence, RI
Undergraduate Research Fellow Jun-Aug 2003
Advisor: Steven Hamburg

- Conducted research in biogeochemistry at Hubbard Brook Experimental Forest and surrounding region; oversaw soil pit excavation by undergraduate and graduate field crew
- Plant surveys, forest floor measurements, litter collection, soil pit excavation, soil physical and chemical analysis, GIS analysis in ESRI ArcMap

PUBLICATIONS IN POPULAR PRESS

- "Extinction Debt,"(Initial author) Wikipedia. Wikimedia Foundation, Inc., February 23, 2011 http://en.wikipedia.org/wiki/Extinction_debt
- “If Everyone Moves to the City, What Gets Left Behind?" *Good.is,* January 17, 2011. http://www.good.is/post/if-everyone-moves-to-the-city-what-is-left-behind/
- “Why the Ethanol Debate Isn’t Helping Anyone,” *GreenBiz.com*, Jun 3, 2009. http://www.greenbiz.com/blog/2009/06/03/why-ethanol-debate-isnt-helping-anyone
- “Four Lean, Green Strategies for an Uncertain Economy,” (with Andrew Shapiro) *Harvard Business Review’s Leading Green*, Oct 29, 2008. http://blogs.hbr.org/2008/10/4-lean-green-strategies-for-an/
- “What a Silent Spring Means for Business Risk,” *GreenBiz.com*, Mar 6, 2007. http://www.greenbiz.com/blog/2007/03/05/what-silent-spring-means-business-risk

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Dollar Amount	Country
211699	CHINA

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

Yes

Is the research exempt from Federal regulations?

No

Does this project involve a clinical trial?

No

G.4.b Inclusion Enrollment Data

Report Attached: Understanding the Risk of Bat Coronavirus Emergence-PROTOCOL-001

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

No

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

No

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional	Address

		District	
Primary: EcoHealth Alliance, Inc.	077090066	NY-010	460 West 34th Street 17th Floor New York NY 100012317
Wuhan Institute of Virology	529027474		Xiao Hong Shan, No. 44 Wuchang District Wuhan
East China Normal University	420945495		3663 Zhongshan Beilu Shanghai
ECOHEALTH ALLIANCE	077090066		ECOHEALTH ALLIANCE, INC. 460 W 34TH ST NEW YORK NY 100012320
EcoHealth Alliance, Inc.	077090066	NY-010	460 West 34th Street 17th Floor New York NY 100012317
Wuhan Institute of Virology	529027474		Xiao Hong Shan, No. 44 Wuchang District Wuhan
East China Normal University	420945495		3663 Zhongshan Beilu Shanghai

G.9 FOREIGN COMPONENT

Organization Name: Wuhan Institute of Virology
Country: CHINA
Description of Foreign Component:
Principal Laboratory for all Research in China as per section G8 (above) and detailed in our Specific Aims

Organization Name: East China Normal University
Country: CHINA
Description of Foreign Component:
Principal Coordinating Team for all project field work as per section G8 (above) and detailed in our Specific Aims

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

Inclusion Enrollment Report

Inclusion Data Record (IDR) #: 166195

Using an Existing Dataset or Resource: No

Delayed Onset Study ?: No

Clinical Trial: No

Enrollment Location: Foreign

NIH Defined Phase III Clinical Trial: No

Study Title: Understanding the Risk of Bat Coronavirus Emergence-PROTOCOL-001

Planned Enrollment

Planned Enrollment Total: 2,460

NOTE: Planned enrollment data exists in the previous format; the PD/PI did not enter the planned enrollment information in the modified format and was not required to do so. Only the total can be provided.

Cumulative Enrollment

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	0	0	0	0	0	0	0	0	**0**
Asian	157	108	0	0	0	0	0	0	0	**265**
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	**0**
Black or African American	0	0	0	0	0	0	0	0	0	**0**
White	0	0	0	0	0	0	0	0	0	**0**
More than One Race	0	0	0	0	0	0	0	0	0	**0**
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	**0**
Total	**157**	**108**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**265**

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 05/26/2017

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI110964-04
FAIN: R01AI110964

Principal Investigator(s):
PETER DASZAK, PHD

Project Title: Understanding the Risk of Bat Coronavirus Emergence

Aleksei Chmura
President
460 West 34th Street
17th Floor
New York, NY 100012317

Award e-mailed to: [redacted] (b) (6)

Period Of Performance:
Budget Period: 06/01/2017 – 05/31/2018
Project Period: 06/01/2014 – 05/31/2019

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $597,112 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to ECOHEALTH ALLIANCE, INC. in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI110964. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Laura A. Pone
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI110964-04

Award Calculation (U.S. Dollars)

Salaries and Wages	$167,708
Fringe Benefits	$54,168
Personnel Costs (Subtotal)	$221,876
Materials & Supplies	$7,000
Travel	$35,918
Other	$9,800
Subawards/Consortium/Contractual Costs	$201,422
Federal Direct Costs	$476,016
Federal F&A Costs	$121,096
Approved Budget	$597,112
Total Amount of Federal Funds Obligated (Federal Share)	$597,112
TOTAL FEDERAL AWARD AMOUNT	$597,112
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$597,112

SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
4	$597,112	$597,112
5	$581,646	$581,646

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name: Allergy and Infectious Diseases Research
CFDA Number: 93.855
EIN: 1311726494A1
Document Number: RAI110964A
PMS Account Type: P (Subaccount)
Fiscal Year: 2017

IC	CAN	2017	2018
AI	8472350	$597,112	$581,646

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M51C / **OC**: 414E / **Released**: [redacted] (b) (6) 05/25/2017
Award Processed: 05/26/2017 12:05:11 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI110964-04

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI110964-04

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget

period.

e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI110964. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:

Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI110964-04

The Research Performance Progress Report (RPPR), Section G.9 (Foreign component), includes reporting requirements for all research performed outside of the United States. Research conducted at the following site(s) must be reported in your RPPR:

San Pya Clinic, BURMA
Institut Pasteur du Cambodge, CAMBODIA
Primate Research Center at Bogor Agricultural University, INDONESIA
Conservation Medicine, Ltd, MALAYSIA
King Chulalongkorn Memorial Hospital, THAILAND
Hanoi Agricultural University, VIETNAM
National Animal Health Laboratory, LAOS

****

This Notice of Award (NoA) includes collaboration with **Wuhan University School of Public Health, CHINA.**

****

This Notice of Award (NoA) includes funds for activity with **Wuhan Institute of Virology, CHINA.**

****

This Notice of Award (NoA) includes funds for activity with **(East China Normal University.**

****

This award may include collaborations with and/or between foreign organizations. Please be advised that short term travel visa expenses are an allowable expense on this grant, if justified as critical and necessary for the conduct of the project.

****

This award is subject to the Clinical Terms of Award included in Monitoring of Clinical Trials and Studies - NIAID (see NIH Guide for Grants and Contracts, July 8, 2002, NOT AI-02-032). These terms and conditions are hereby incorporated by reference, and can be accessed via the following World Wide Web address: https://www.niaid.nih.gov/grants-contracts/niaid-clinical-terms-award All submissions required by the NIAID Clinical Terms of Award must be forwarded electronically or by mail to the responsible NIAID Program Official identified on this Notice of Award.

****

Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- o A list of the new and/or additional Agent(s) that will be studied;
- o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Carine Normil
Email: [redacted] (b) (6) **Phone**: [redacted] (b) (6) **Fax**: 301-493-0597

Program Official: Erik J. Stemmy
Email: [redacted] (b) (6) **Phone**: [redacted] (b) (6)

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI110964-04

INSTITUTION: ECOHEALTH ALLIANCE, INC.

Budget	Year 4	Year 5
Salaries and Wages	$167,708	$167,708
Fringe Benefits	$54,168	$54,168
Personnel Costs (Subtotal)	$221,876	$221,876
Materials & Supplies	$7,000	$3,500
Travel	$35,918	$35,918
Other	$9,800	$9,400
Subawards/Consortium/Contractual Costs	$201,422	$191,576
TOTAL FEDERAL DC	$476,016	$462,270
TOTAL FEDERAL F&A	$121,096	$119,376
TOTAL COST	$597,112	$581,646

Facilities and Administrative Costs	Year 4	Year 5
F&A Cost Rate 1	44.1%	44.1%
F&A Cost Base 1	$274,594	$270,694
F&A Costs 1	$121,096	$119,376

A. COVER PAGE

Project Title: Understanding the Risk of Bat Coronavirus Emergence	
Grant Number: 5R01AI110964-04	**Project/Grant Period:** 06/01/2014 - 05/31/2019
Reporting Period: 06/01/2016 - 05/31/2017	**Requested Budget Period:** 06/01/2017 - 05/31/2018
Report Term Frequency: Annual	**Date Submitted:** 04/12/2017
Program Director/Principal Investigator Information: PETER DASZAK , BS PHD **Phone number:** (b) (6) **Email:** (b) (6)	**Recipient Organization:** ECOHEALTH ALLIANCE, INC. ECOHEALTH ALLIANCE, INC. 460 W 34TH ST 17TH FLOOR NEW YORK, NY 100012320 **DUNS:** 077090066 **EIN:** 1311726494A1 **RECIPIENT ID:**
Change of Contact PD/PI: N/A	
Administrative Official: ALEKSEI CHMURA 460 W 34th St., 17th Floor New York, NY 10001 **Phone number:** (b) (6) **Email:** (b) (6)	**Signing Official:** ALEKSEI CHMURA 460 W 34th St., 17th Floor New York, NY 10001 **Phone number:** (b) (6) **Email:** (b) (6)
Human Subjects: Yes HS Exempt: No Exemption Number: Phase III Clinical Trial:	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Zoonotic coronaviruses are a significant threat to global health, as demonstrated with the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, and the recent emergence Middle East Respiratory Syndrome (MERS-CoV). The wildlife reservoirs of SARS-CoV were identified by our group as bat species, and since then hundreds of novel bat-CoVs have been discovered (including >260 by our group). These, and other wildlife species, are hunted, traded, butchered and consumed across Asia, creating a largescale human-wildlife interface, and high risk of future emergence of novel CoVs.
To understand the risk of zoonotic CoV emergence, we propose to examine 1) the transmission dynamics of bat-CoVs across the human-wildlife interface, and 2) how this process is affected by CoV evolutionary potential, and how it might force CoV evolution. We will assess the nature and frequency of contact among animals and people in two critical human-animal interfaces: live animal markets in China and people who are highly exposed to bats in rural China. In the markets we hypothesize that viral emergence may be accelerated by heightened mixing of host species leading to viral evolution, and high potential for contact with humans. In this study, we propose three specific aims and will screen free ranging and captive bats in China for known and novel coronaviruses; screen people who have high occupational exposure to bats and other wildlife; and examine the genetics and receptor binding properties of novel bat-CoVs we have already identified and those we will discover. We will then use ecological and evolutionary analyses and predictive mathematical models to examine the risk of future bat-CoV spillover to humans. This work will follow 3 specific aims:

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces. We will examine if: 1) wildlife markets in China provide enhanced capacity for bat-CoVs to infect other hosts, either via evolutionary adaptation or recombination; 2) the import of animals from throughout Southeast Asia introduces a higher genetic diversity of mammalian CoVs in market systems compared to within intact ecosystems of China and Southeast Asia; We will interview people about the nature and frequency of contact with bats and other wildlife; collect blood samples from people highly exposed to wildlife; and collect a full range of clinical samples from bats and other mammals in the wild and in wetmarkets; and screen these for CoVs using serological and molecular assays.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk. We propose two competing hypotheses: 1) CoV host-range in bats and other mammals is limited by the
phylogenetic relatedness of bats and evolutionary conservation of CoV receptors; 2) CoV host-range is limited by geographic and ecological opportunity for contact between species so that the wildlife trade disrupts the 'natural' co-phylogeny, facilitates spillover and promotes viral evolution. We will develop CoV phylogenies from sequence data collected previously by our group, and in the proposed study, as well as from Genbank. We will examine co-evolutionary congruence of bat-CoVs and their hosts using both functional (receptor) and neutral genes. We will predict host-range in unsampled species using a generalizable model of host and viral ecological and phylogenetic traits to explain patterns of viral sharing between species. We will test for positive selection in market vs. wild-sampled viruses, and use data to parameterize mathematical models that predict CoV evolutionary and transmission dynamics. We will then examine scenarios of how CoVs with different transmissibility would likely emerge in wildlife markets.

Specific Aim 3: Testing predictions of CoV inter-species transmission. We will test our models of host range (i.e. emergence potential) experimentally using reverse genetics, pseudovirus and receptor binding assays, and virus infection experiments in cell culture and humanized mice. With bat-CoVs that we've isolated or sequenced, and using live virus or pseudovirus infection in cells of different origin or expressing different receptor molecules, we will assess potential for each isolated virus and those with receptor binding site sequence, to spill over. We will do this by sequencing the spike (or other receptor binding/fusion) protein genes from all our bat-CoVs, creating mutants to identify how significantly each would need to evolve to use ACE2, CD26/DPP4 (MERS-CoV receptor) or other potential CoV receptors. We will then use receptor-mutant pseudovirus binding assays, in vitro studies in bat, primate, human and other species' cell lines, and with humanized mice where particularly interesting viruses are identified phylogenetically, or isolated. These tests will provide public health-relevant data, and also iteratively improve our predictive model to better target bat species and CoVs during our field studies to obtain bat-CoV strains of the greatest interest for understanding the mechanisms of cross-species transmission.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: 5R01AI110964-04.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: 5R01AI110964-04 Professional Development.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

1.Conference and University Lectures: PI Daszak, and Co-investigators Shi, Epstein, Olival, and Zhang gave invited University and Conference lectures including Avoiding Catastrophe Meeting at Concordia Univ., Harvard Univ. Columbia Univ., National Academy of Sciences, World Humanitarian Summit in Turkey, NEIDL Symposium in Boston, Global Pandemic Policy Summit at Texas A&M Univ., One Health EcoHealth Congress in Australia, WHO briefing, Rockefeller Planetary Health meeting, 17th International Bats Conference, China National Global Virome Project Initiative Meeting, and others that included specific discussion of the current project and results.

2.Agency and other briefings: PI Daszak and Co-investigator Shi introduced this project to potential collaborators within Rockefeller Foundation, WHO, FAO, International Collaboration Bureau of Chinese Academy of Sciences, Beijing Genomic Institute, National Natural Science Foundation of China, Institute of Pathogen Biology, Chinese Academy of Medical Science & Peking Union Medical College, and Chinese CDC.

3.Public outreach: PI Daszak and Co-investigator Shi presented this work to members of NSF, NIH, U.S. CDC, the State of Forestry Administration of China, and the general public at the China National Virome Project Initiative Meeting hosted by Chinese CDC and Chinese Academy of Sciences (2017); Co-investigator Olival presented this work at the NYC Medtech Forum to the public (2016); Research Technician Dr. Guangjian Zhu presented this work at the China Conservation Expo to the conservation groups in China (2016). Co-Investigator Y-Z Zhang presented this project to the provincial infectious disease hospital Kunming No.3 People's Hospital in Yunnan province (2016).

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces.

•To commence the analysis of data collected from the integrated biological behavioral surveillance questionnaires from Yunnan, Guangxi, and Guangdong provinces, linking to the viral and serological testing results of biological samples.

•Following the successful pilot of wildlife trade network research in Lipu, Guilin, Guangxi province in Year 3, we will continue the Wild Animal Farms Survey in Guangxi, and expand to Yunnan and Guangdong in Year 4, with Institutional Review Board approvals from both Yunnan Institute of Endemic Diseases Control and Prevention and Hummingbird #2016-55, to:

−Generate a network model of wildlife trade
−Model trade flows in the wildlife farmer networks to identify locations of high potential for viral recombination
−Update survey instrument for "second wave" network interviews

•We will continue the passive hospital surveillance with anonymized, surveillance data collection from acutely ill hospital in-patients who 1) satisfy syndromic eligibility criteria; 2) have complete medical records; 3) non-normative laboratory confirmed diagnostic results; and suspected acute viral infection.

Research has been successfully piloted in four hospitals in Yunnan province: 1) Dali College Affiliated Hospital; 2) Dali Prefecture Hospital; 3) Kunming No. 3 People's Hospital, and 4) Chuxiong Prefecture Hospital, 120 biological samples have been collected, with approval from the Institutional Review Boards of the School of Public Health of Wuhan University and Hummingbird IRB

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk

•The genomic characterization of SL-CoVs in Year 3 was focused on Rhinolophus sinicus in Yunnan, our plan for Year 4 is to obtain complete S gene, RdRp gene or full-length genome sequences of more SL-CoVs from a broader range of bat species identified all over China and conduct a more comprehensive evolution study on SL-CoVs in bats.

•To search for the receptor of SL-CoV with deletions in the homologous region of SARS-CoV RBD (i.e. Rp3, Rs672), and SL-CoVs which has been demonstrated to be unable to utilize bat ACE2 (i.e. Rs4231) whose receptors may be some molecules other than ACE2.

•To conduct population genetics study of Rhinolophus sinicus ACE2s, which includes: the amplification of ACE2 genes from Rhinolophus sinicus samples of different origin, test of the usage efficiency of Rhinolophus sinicus ACE2s of different origins by SL-CoVs and kinetics study on the binding of SL-CoV RBD to different Rhinolophus sinicus ACE2s.

•Phylogeographic study of bat-CoV to better understand the geographic distribution and evolution of bat-CoV genetic diversity in south China.

•Phylogeographic study of bat host (Rhinolophus) species to assess the connectivity of bat populations and infer their historical movements and demographic history to improve our understanding of CoV transmission among bat populations in southern China.

•Cophylogenetic analyses of bat host and CoV phylogenies to assess frequency of cross-species transmission. Comparison of Alpha- and Beta-CoV cophylogenetic patterns building on Year 3 analyses using published sequences.

Specific Aim 3: Testing predictions of CoV inter-species transmission.

•Using the reverse genetic method, we will construct chimeric viruses with the backbone of MERS-CoV and the S genes from diverse newly identified bat MERS-related coronaviruses, and examine the pathogenicity of bat MERS-related coronaviruses on cell and animal levels.

•The animal infection experiments are planned to be conducted in following years to study the pathogenicity of diverse SL-CoVs and MERS-related CoV that we identified in Chinese bats.

•Surveillance of infection in human populations by bat-borne CoVs in Guangxi and Guangdong provinces in previously identified areas with human populations of high risk of exposure to bats. PCR and ELISA will be used, respectively, for detection of viral nucleic acids and antibodies against the viral nucleocapsid protein or spike protein.

Year 3 Report: Understanding the Risk of Bat Coronavirus Emergence

Award Number: 5R01AI110964-04

B.2 What was accomplished under these goals?

SUMMARY

The results of the 3rd year of our R01 work are detailed below. They include:

- Initial analysis of behavioral risk qualitative research in Yunnan and wildlife market observational data in Guangdong, that suggests a reduction in wildlife hunting, trade and consumption may be underway in southern China.
- Results from a behavioral risk survey of over 1,000 people in two provinces of southern China that assesses exposure to wildlife and prior bouts of unusual illness, with concurrent taking of samples to test for evidence of exposure to SL-CoVs.
- The finding of serological evidence of spillover of bat SARS-like CoVs in 6 people in Yunnan
- Testing of over 1,000 bat samples to identify diverse alpha- and betacoronaviruses
- Full genome characterization of 26 alphacoronaviruses.
- Receptor binding domain sequences from 37 new bat SL-CoVs that shows S proteins re more diverse than previously thought.
- Host-virus co-phylogeographic analysis of a diverse group of >1,300 bat CoVs showing that these viruses have a larger host range, weaker host specificity and higher frequency of cross-genera transmission than previously thought.
- Use of our reverse genetics system to identify 3 more novel SL-CoVs with potential to directly infect people.

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces

During Year 3 we began analyzing the qualitative research that was conducted in Year 2. In addition, we developed a digital application for a community-based integrated biological behavioral surveillance system and rolled this out in two provinces. The tool aims to identify specific animal exposure risk factors associated with biological evidence of exposure to SARS-like CoV (i.e. seropositive status).

Qualitative Research

Interviews conducted in Yunnan province during Year 2 were transcribed and translated into English. A total of 23 individuals (12 women; 11 men) were interviewed in rural regions where wildlife trade routes have been documented. Yunnan province was specifically selected for study because they have large wildlife populations, a diversity of wildlife species and numerous live animal markets. Individuals who were 18 years of age or older and who were able to provide informed consent were eligible to participate. The study was approved by the Institutional Review Boards of the School of Public Health at Wuhan University and Hummingbird IRB #2014-23.

Participants were recruited primarily through local contacts that have been developed as part of wildlife conservation and health research that has been ongoing in these regions in China for the past decade. Contacts including wildlife conservationists and researchers, local government health outreach workers and wildlife farmers facilitated introductions and provided referrals. To achieve a sample with sufficient representation of categories of interest, participants were recruited using purposive sampling, which provides minimum quotas in terms of sex, age and wildlife exposure setting (e.g., live animal market, forest preserve).

Educational attainment varied widely in the population; however, the majority of study participants reported limited schooling, primary education or less. This was further reflected in the occupational distribution of study participants (*Fig. 1*), while there was two respondents who reported more professional occupations, a doctor and an accountant, half (50%) were unskilled laborers or farmers, either agricultural or animal. There were one individuals who self-identified as animal farmers, farming wildlife, bamboo rat, civet, or nutria.

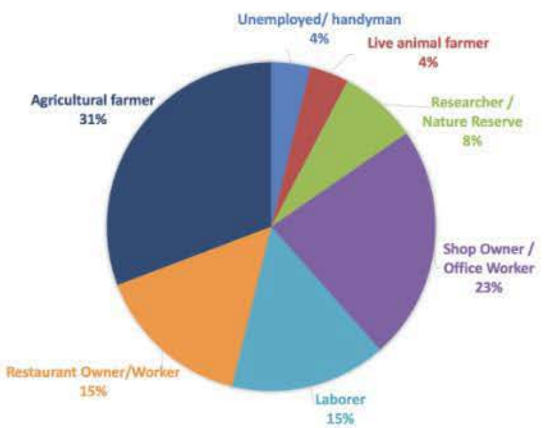


Figure 1. Occupation of Qualitative Research Participants (n=23) in Yunnan and Guangxi Provinces

Thematic analysis provided the framework with which to code and analyze data from the ethnographic interviews and focus group. Five core themes were identified to form the basis for this: (1) human-animal contact, (2) unusual illness experience and response, (3) socioeconomics and daily living, (4) biosafety and (5) human environments and movement/travel. Individual interviews and field notes were studied to ensure familiarity with the data set in its entirety and to confirm narrative consistency within individual interviews prior to coding. Using these themes and a coding keyword guide allowed for a directed and consistent coverage of the domains that were the focus of the actual interviews. Qualitative data were re-examined to develop additional theoretical categories or typologies. This analysis aims to assess perceptions, knowledge and participation in the wildlife trade, as well as barriers to participation and observed changes over time. The data were coded for factors associated with wildlife consumption, socioeconomic drivers of wildlife trade, conservation and legal efforts, the prevalence and types of wildlife observed, and wildlife exposures that could transmit disease to humans (*Table 1*).

Table 1. Topics covered in Ethnographic Interviews

Theme Discussed in Ethnographic Interview	No. of Respondents (n=23)	(%)
Work/Job Functions	22	96%
Water & Food	22	96%
Sanitation	22	96%
Hygiene	22	96%
Perceptions/Knowledge	22	96%
Home Life	21	91%
Education	20	87%
Medical Care Treatment	20	87%
Direct Contact with Animals	20	87%
Travel	19	83%
Observed Environment	19	83%
Animal Responsibilities	19	83%
Household Illness	19	83%
Indirect Contact with Animals	19	83%
Daily Routine	18	78%
Family Economics	18	78%
Illness from Animals	18	78%
Animal Health	18	78%
Animal Products/Rituals with Animal Products	16	70%
Death	14	61%

The data coding and analytic strategy was designed to avoid the need for expensive analytic software programs and to use standard word processing and spreadsheet programs readily available to in-country teams. These teams received training on qualitative data analysis, and they initiated the first phase of analyses.

Analysis focused on wildlife trade and consumption in these two provinces, specifically on how respondents perceive and contact wildlife through the changing landscape around them. The aim was to identify motivations around animal consumption and practices. A number of participants reported that wildlife are purchased as a means to impress others as a symbol of wealth. Participants routinely reported that the cost of wildlife is double or triple that of regular livestock meat. Ironically, others reported that poorer individuals in these communities who continue to eat wildlife are sometimes scorned for their poverty, because this is a habit from an older time within China. Though there is a stigma to this habit, individuals did report opportunistically capturing and consuming wildlife when convenient.

Participants also noted a decrease in wildlife over time: that in their childhood the forests would be full of the sounds of animals and birds, but this occurs no longer. This decrease was attributed to many factors, most commonly infrastructure development. Respondents discussed

the government investing resources to build new roads and renovate local infrastructure with the intention of increasing tourism, and that this has had the impact of reducing forested habitat for wildlife. Hunting and selling of wildlife was not reported by any participant as a cause of observed wildlife depletion. However, participants did attribute a reduction in wildlife hunting and consumption to an increased enforcement of conservation laws. In particular, the story of one ill-fated hunter who killed a monkey—and was caught—was reported by a number of participants from the same village.

Participants observed that the observed decrease in wildlife abundance and increased conservation law enforcement has made it more difficult to make a living from the wildlife trade. Participants reported choosing alternative forms of money making, indicating that only those people who belong to low socioeconomic classes continue to hunt secretly. The cost-benefit analysis that pits the threat of punitive consequences against the profits to be made through wildlife hunting are only feasible for those 'who have nothing to lose.'

Table 2: Species Observed in Wet Markets in Guangdong Province from 2015 - 2016

Genus species	Common Name
Prionailurus bengalensis	Leopard Cat
Nyctereutes procyonoides	Raccoon Dog
Sus scrofa	Wild Boar
Lepus sinensis	Chinese Hare
Arctonyx collaris	Hog Badger
Hystrix brachyura	Porcupine
Marmota sp.	Marmot
Rhizomes sinensis	Bamboo Rat
Erinaceus sp.	Hedgehog
Mustela putorius	Ferrets
Muridae	Rat (species unknown)
Myocastor coypus	Nutria
Vulpes sp.	Fox
Mustela sibirica	Siberian weasel
Paguma larvata	Masked Palm Civet
Felis catus	Domestic Cat
Canis lupus familiaris	Domestic Dog
Cervinae	Sambar Deer
Ovis aries	Sheep
Capra sp.	Domestic Goat
Rattus norvegicus	Common Rat

Observations by research staff in live animal markets in Guangzhou found wildlife to be plentiful (*Table 2*), although no bats were seen for sale during the observation period. In contrast, wildlife

was not found in live animal markets at the sites we visited in either Yunnan or Guangxi. This is a change from previous research visits to the same or similar communities, when bats, rodents and wild boar could be found. Locals in Yunnan and Guangxi attribute the change to conservation law enforcement. The success of conservation enforcement may have moved hunting and trapping underground and made the capture of local wildlife less economically feasible than other income generating activities.

Integrated Biological Behavioral Surveillance in Yunnan and Guangdong Provinces
To better assess the mechanisms of zoonotic viral spillover, and build on data acquired via ethnographic interview (above) we have designed a structured behavioral questionnaire to measure both exposure and outcome data. This behavioral risk survey assesses exposure to wildlife and bouts of unusual illness over a respondent's lifetime and in the past 12 months. In addition, participants were requested to provide serum to test for previous exposure to SARS-like CoV. The integrated surveillance was pilot-tested in October 2015 among residents living near bat caves or roosts where SL-CoVs have been previously detected in the bat population in Jinning County, Yunnan. After the questionnaire was pilot tested and optimized to fit the research aim, the survey was developed as a digital application (https://www.dropbox.com/s/sv62neywuvl027r/Questionnaire%20Complete.docx?dl=0%). This allows standardization across all field teams and quality control. Four field team leads were trained on behavioral survey data collection, data collection technologies (the digital application) and analysis. The questionnaire was then administered in a follow-up survey in Yunnan province and then in Guangdong province. Surveillance in Guangxi is currently underway.

Of 1089 participants who completed the behavioral questionnaire, 660 (61%) were women and 424 (39%) were men (5 missing for this variable), with a mean age of 50 (range: 10-99). Most reported being farmers (79%) (*Fig. 2*), a majority were long term residents (97%) and 41% had a family income under 3000 RMB annually ($430). Almost three quarters (72%) of the respondents have had only primary level education or less.

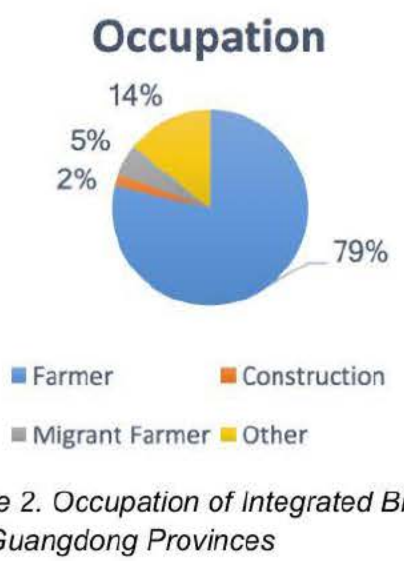


Figure 2. Occupation of Integrated Biological Behavioral Surveillance Participants in Yunnan and Guangdong Provinces

Standardized syndromic case definitions informed questions concerning unusual illness experience (e.g. severe acute respiratory infections [SARI], influenza-like illness [ILI], febrile symptoms [Encephalitis]). Lifetime, 12 month, and unusual illnesses experienced in the family for the past 12 months were assessed for all participants. In the past year, SARI was reported by 55 (5.1%) respondents and 14 of those respondents also responded SARI symptoms in family members (*Table 3*).

Table 3. Unusual Illness Experience In Respondents Lifetime, Past 12 months, Family members

Symptoms	Ever	Past 12 months	Family Past 12 months
Severe Acute Respiratory Infections (SARI)	118 (10.8%)	55 (5.1%)	40 (3.7%)
Influenza Like Illness (ILI)	305 (28.0%)	128 (11.8%)	142 (13.0%)
Encephalitis	98 (9.0%)	52 (4.8%)	30 (2.8%)
Hemorrhagic Fever	2 (0.2%)	2 (0.2%)	0 (0.0%)
Fever with Diarrhea /Vomiting	58 (5.3%)	25 (2.3%)	21 (1.9%)
Fever with Rash	10 (0.9%)	7 (0.6%)	7 (0.6%)

Type of exposure and species exposed to are shown below (*Table 4*). Poultry was the most commonly contacted animal in almost all categories. Three quarters of respondents reported rodents or shrews entering their home in the past 12 months.

Table 4: Animal Species Contact by Type of Contact

	Pets	Handled	Raised	In house	Cooked/ handled	Eaten raw/ under-cooked	Found dead collected	Scratched/ bitten	Slaughtered	Hunted/ trapped
Rodents/Shrews	0	33	5	834	38	2	1	1	28	26
Bats	0	5	0	180	8	0	0	1	5	5
Non-human primates	0	1	3	7	4	0	0	0	1	1
Birds	3	19	8	497	39	3	0	0	12	12
Carnivores	1	16	7	100	36	0	0	0	19	10
Ungulates	0	5	12	23	50	0	0	0	8	1
Poultry	5	514	843	134	719	5	8	6	425	7
Goats/Sheep	0	16	38	4	80	1	0	0	17	0
Swine	3	210	494	43	533	47	1	1	147	2
Cattle/Buffalo	0	12	77	10	102	5	1	0	11	1
Dogs	342	40	303	252	62	0	0	22	16	2
Cats	163	10	137	275	18	0	0	11	1	0

Animal exposures among those who reported unusual illness experiences in the past 12 months were evaluated, focusing on three high interest syndromes: SARI, ILI, and encephalitis. Of the 55 respondents who reported SARI symptoms, 49 reported: raising animals; animals in the home; preparing recently killed animals and buying live animals; 50% reported slaughter. Among the 16 respondents who reported ILI symptoms, 12 (75%) reported handling/preparing recently killed animals, 11 (69%) handling live animals or having animals in the home, 10 (63%) reported slaughtering/killing animals or buying live animals at wet market, 9 (56%) raised live animals, 7 (44%) reported a pet, and 1 (6%) reported animal feces near food or eating animal touched or damaged food, hunting, or eating raw/undercooked animal products. Among the four respondents who reported encephalitis symptoms, 3 (75%) reported hunting, handling or raising animals, 2 (50%) reported animals in the home, 1 (25%) reported having animals as pets, slaughtering/killing animals, or having bought live animals at a wet market.

Table 5. Self-Reporting Symptoms of Syndromes and Sociodemographic and Animal Contact.

	SARI Positive *n=55*		**ILI Positive** *n=128*		**Encephalitis Positive** *n=52*	
Sociodemographics	n	%	n	%	n	%
Mother Primary education or less	54	98.2%	121	94.5%	50	96.2%
Primary education or less	45	81.8%	94	73.4%	38	73.1%
Female	32	58.2%	74	57.8%	29	55.8%
Income <3000RMB	30	54.5%	45	35.2%	23	44.2%
Travel (past 12m)	30	54.5%	69	53.9%	34	65.4%
Children < 5 yrs in Household	15	27.3%	38	29.7%	17	32.7%
Household member with same syndrome	14	25.5%	46	35.9%	10	19.2%
Respondent age <35	6	10.9%	24	18.8%	14	26.9%
Animal Exposures	n	%	n	%	n	%
Come in home	50	90.9%	117	91.4%	50	96.2%
Raise animals	49	89.1%	113	88.3%	48	92.3%
Prepare/cook recently killed	37	67.3%	95	74.2%	35	67.3%
Handle live	36	65.5%	72	56.3%	38	73.1%
Slaughtered	31	56.4%	57	44.5%	34	65.4%
Animals as Pets	23	41.8%	55	43.0%	28	53.8%
Buy Animals at Wet Market	16	29.1%	49	38.3%	4	7.7%
Shared water source	9	16.4%	13	10.2%	12	23.1%
Feces in/near food	8	14.5%	9	7.0%	8	15.4%
Consume raw/undercooked	7	12.7%	10	7.8%	9	17.3%
Scratch/bite	4	7.3%	2	1.6%	4	7.7%
Consume food damaged by animals	3	5.5%	5	3.9%	2	3.8%
Hunt or Trap	2	3.6%	4	3.1%	7	13.5%
Collect dead wildlife	1	1.8%	1	0.8%	1	1.9%
Consume sick animals	0	0.0%	1	0.8%	0	0.0%

We examined the sociodemographic attributes and the types of contacts that were reported in those who reported SARI, ILI, or encephalitis-like symptoms in the past year (see *Table 5*). Over 65% of respondents these syndromes and also reported raising animals, animals coming in the home, or preparing meat or organs from a recently killed animal. A quarter of those who reported symptoms consistent with that of encephalitis were under the age of 35.

Respondents were asked about the source of their unusual illnesses. None reported any kind of animal exposure as a potential source of infection and 11% did not have any idea what may have caused their previous infection, despite the fact that a majority of respondents who reported SARI, ILI, or encephalitis symptoms also reported animal exposures (Table 5). Just over 30% of respondents reported purchasing live animals from a wet market in the past year. Over half (582; 53%) of respondents were worried about disease or disease outbreaks in animals at wet markets and 56% of people believe that animals spread disease. However, those who had purchased animals from markets in the last 12 months reported a great deal of behavior change being undertaken. In particular, respondents reported buying live animals less often 33%, only buying farmed wildlife 32% or buying meat at the supermarket 30% (*Table 6*). For those who participated in animal slaughter or were scratched or bitten in the past year, only 48 respondents (9.9%) reported visiting a doctor.

Table 6: Behavior Change at Wet Market in the last 12 months

Behavior	n	%
Wash hands	119	33.4%
Buy live animals less often	119	33.4%
Buy only farmed wildlife	113	31.7%
Sometimes shop for meat at supermarket	107	30.1%
Wear gloves	7	1.9%
Wear a mask	5	1.4%

Serological Evidence of Bat SARS-Like CoV Infection in Humans

Along with the behavioral survey questionnaire, respondents were also asked to provide a biological sample to assess SARS CoV spillover at the high-risk location where the questionnaire has been implemented.

A sensitive and specific ELISA method was developed using the recombinant bat SL-CoV Rp3 NP protein to detect SL-CoV IgG antibodies. Six (2.8%) serum samples from 218 village residents who lived closely to the bat colonies in Yunnan where we isolated SL-CoV WIV1 and WIV16 were positive for SARS-like CoV antibodies (*Fig. 3*). The 6 ELISA positive samples were further confirmed as anti-SL-CoV NP IgG positive by western blot using recombinant Rp3-NP as antigen (*Fig. 4*).

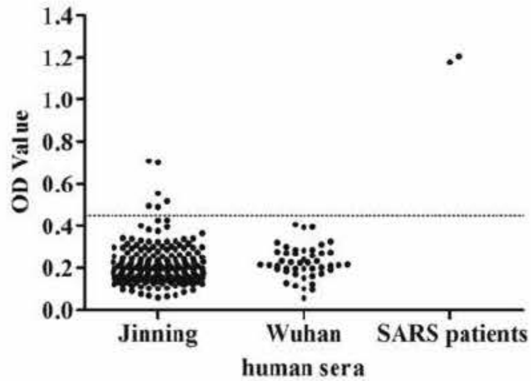


Figure 3. Serum samples from Jinning, Wuhan, and SARS patients were screened for reactivity of Rp3-NP. Bar in the diagram indicates optical density (OD) cutoff value (0.45) based on healthy blood donors in Wuhan.

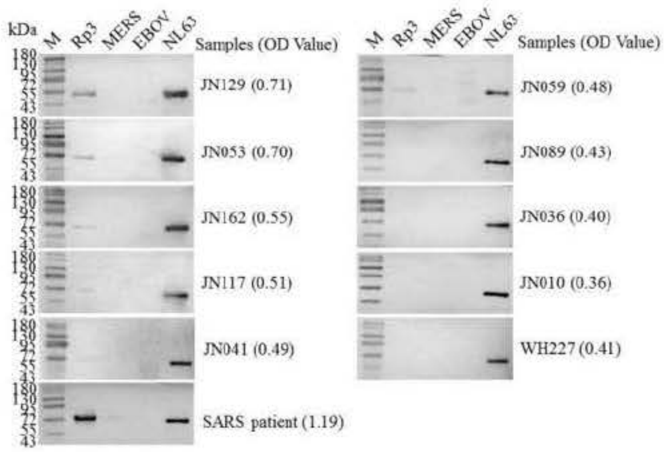


Figure 4. Western blot analysis of reactivity of human sera to Rp3-NP.

Linking Serological Findings with Respondent Questionnaire Data

Of the 6 respondents in Jinning, Yunnan with serological evidence of SL-CoV infection, 4 had handled animals, 3 had raised or cooked meat from recently killed animals, 2 found animal feces near food stuffs, and 1 slaughtered or hunted an animal. Three of the individuals had contact with poultry in the past twelve months and 2 had contact with either birds, swine or buffalo. One individual reported having contact with a bat. Responses to the questionnaire show that in the last twelve months all of the respondents who have positive testing results, had animals in their dwelling and had contact with rodents or shrews. All 6 of the respondents had reported purchasing an animal from a wet market in the past twelve months.

In addition, 215 oral swabs and 212 rectal swabs collected from human participants in Jinning and Yunnan province were tested for CoV RNA, and no positive results were found. 534 oral swabs, 526 rectal swabs from Xishuangbanna, Yunnan province; and 419 oral swabs, 412 rectal swabs from Ruyuan and Zengcheng, Guangdong province are being tested for CoV.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV spillover risk

Bat CoV PCR Detection and Sequencing from Live-Sampled Bat Populations

We collected 893 rectal swab samples, 167 fecal samples and 33 blood samples from at least 17 bat genera in Yunnan, Guangdong, Guangxi, Hubei and Tibet provinces (*Table 7*) in Year 3. During this year, overall 1060 samples were tested for CoV RNA and 130 (12.3%) were positive (*Table 8*).

Table 7. Bat samples collected for CoV surveillance in Year 3

Date of Sampling	Sampling Locations	Rectal swab	Fecal pellet	Blood specimen
May 11th 2016	Mengla, Yunnan	32	--	9
May 16th 2016	Jingna, Yunnan	16	114	13
May 22nd 2016	Lufeng, Yunnan		53	--
June-July, 2016	Shixing county, Shaoguan, Guangdong	113	--	--
July 2016	Qingzhangshan, Shaoguan, Guangdong	101	--	--
July 10th 2016	Ruyuan, Guangdong	16	--	--
July 11th 2016	Chengjia, Nanling, Guangdong	26	--	--
July 2016	Huadu, Guangzhou, Guangdong	29	--	--
August 6th 2016	Lengshuitang village, Guilin, Guangxi	135	--	--
August 6th 2016	Nanxishan Park, Guilin, Guangxi	31	--	--
August 9th 2016	Lanwu village, Ruyuan, Guangdong	53	--	--
August 10th 2016	Liangkou twon, Conghhua, Guangdong	32	--	--
August 13th 2016	Jinning, Yunnan	34	--	--
August 14th 2016	Lufeng, Yunnan	25	--	--
August 16th 2016	Jingna, Yunnan	33	--	--
August, 2016	Menghai, Yunnan	125	--	--
August 21st 2016	Yaoqu village, Mengla, Yunnan	30	--	--
September, 2016	Wuhan, Hubei	36	--	--
September, 2016	Motuo, Tibet	26	--	11
Total		893	167	33

Genetically diverse alphacoronaviruses related to bat coronavirus 1A/1B, HKU7, HKU6 and HKU2 were identified in *Miniopterus*, *Myotis* and *Rhinolophus* bats, respectively. A novel alphacoronavirus related to human coronavirus NL63 was detected in *Tylonycteris robustula* in Yunnan. SARS-like coronaviruses were detected in 14 Chinese horseshoe bats (*Rhinolophus sinicus*) in Yunnan and Guangdong. Betacoronaviruses related to HKU5 were found in *Pipistrellus abramus* from Hubei, while two lineages of HKU4-related viruses were identified in two species of *Tylonycteris* bats in Yunnan (*Fig. 5*).

Table 8. CoV testing results for bat samples collected in Year 3

Species	Yunnan	Guangdong	Guangxi	Hubei	Tibet	Total
Rousettus spp.	1/34				6	1/40
Aselliscus stoliczkanus	31					31
Rhinoluphus spp.	16/41	11/136	6/60		5	33/242
Hipposideros spp.	17	1/126	6		8	1/157
Myotis spp.	7	6/34	7/69	1		13/111
Chaerephon spp.	8					8
Megaderma spp.	2				1	3
Ia io	1					1
Tylonycteris spp.	32/124	8				32/132
Pipistrellus spp.	1	45		5/35	2	5/83
Eonycteris spelaea	1/29					1/29
Nyctalus velutinus		2				2
Coelops spp.		2				2
Miniopterus spp.		9/17				9/17
Taphozous melanogopon			31			31
Cynopterus sphinx					3	3
Murina spp.					1	1
Fecal pellets	35/167					35/167
Sub-total	**85/462**	**27/370**	**13/166**	**5/36**	**0/26**	**130/1060**

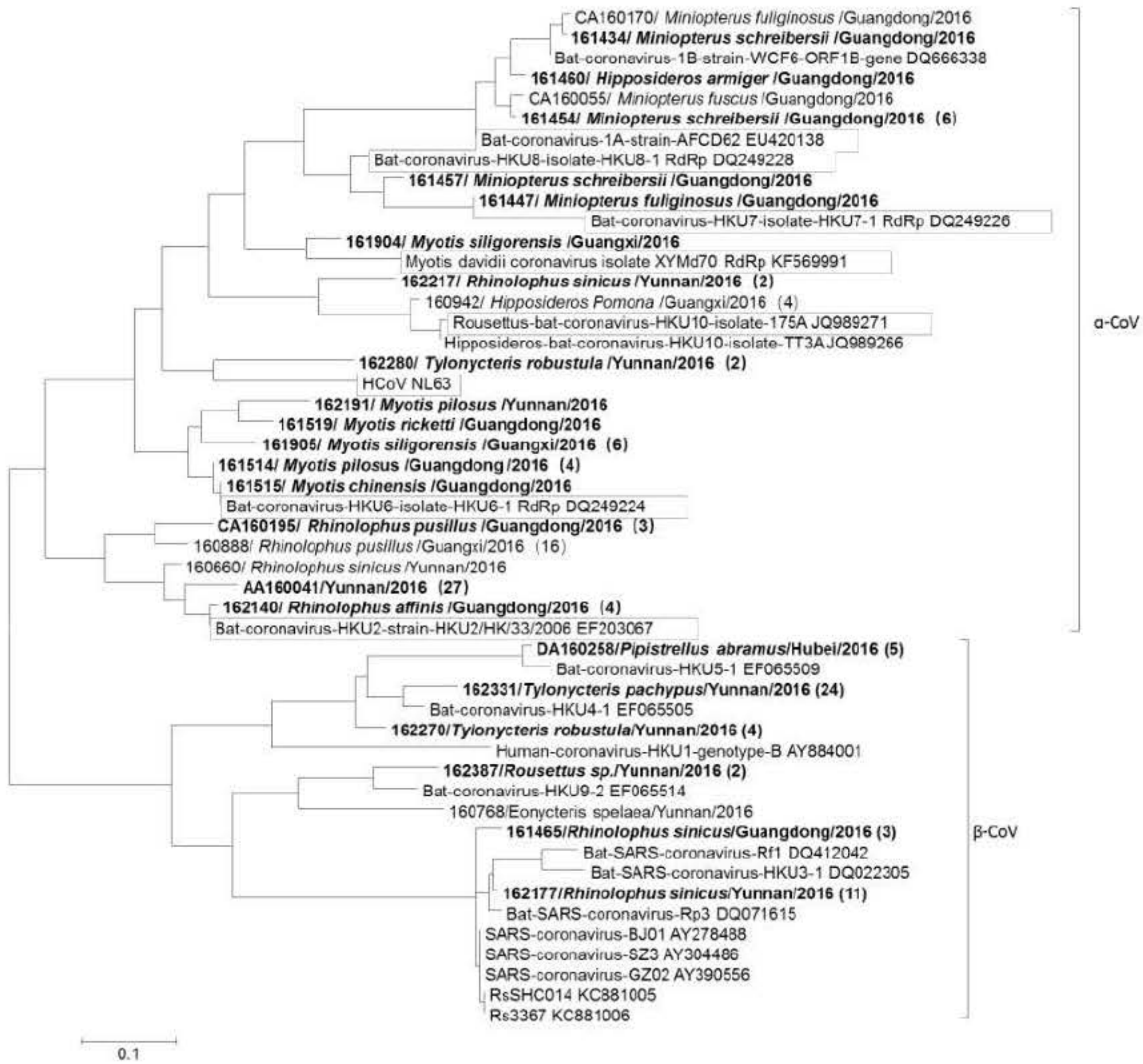


Figure 5. Phylogenetic analysis of partial RdRp gene of CoV (440-nt partial sequence).

Genomic Characterization of Novel Bat Alpha- Coronaviruses

We generated full-length genome sequences of 26 novel alphacoronaviruses from multiple *Hipposidoeros*, *Rhinolophus* and *Hypsugo* bat species. These alphacoronaviruses grouped into 4 different lineages, including HKU10-like CoVs and 3 novel species according to criteria generated by the International committee of Taxonomy of Viruses (ICTV) (*Fig. 6*). Strains belonging to the novel lineage from *Rhinolophus* share highly similar genome structures with each other but are distinct from all previously sequenced alphacoronaviruses. Putative 3b and 3c genes were identified at the upstream of the E gene, and a 7b gene at the downstream of the N gene was a homologue to *Rhinolophus* bat SARS-like CoV 7a gene. These results expand the understanding of genetic diversity of bat alphacoronaviruses.

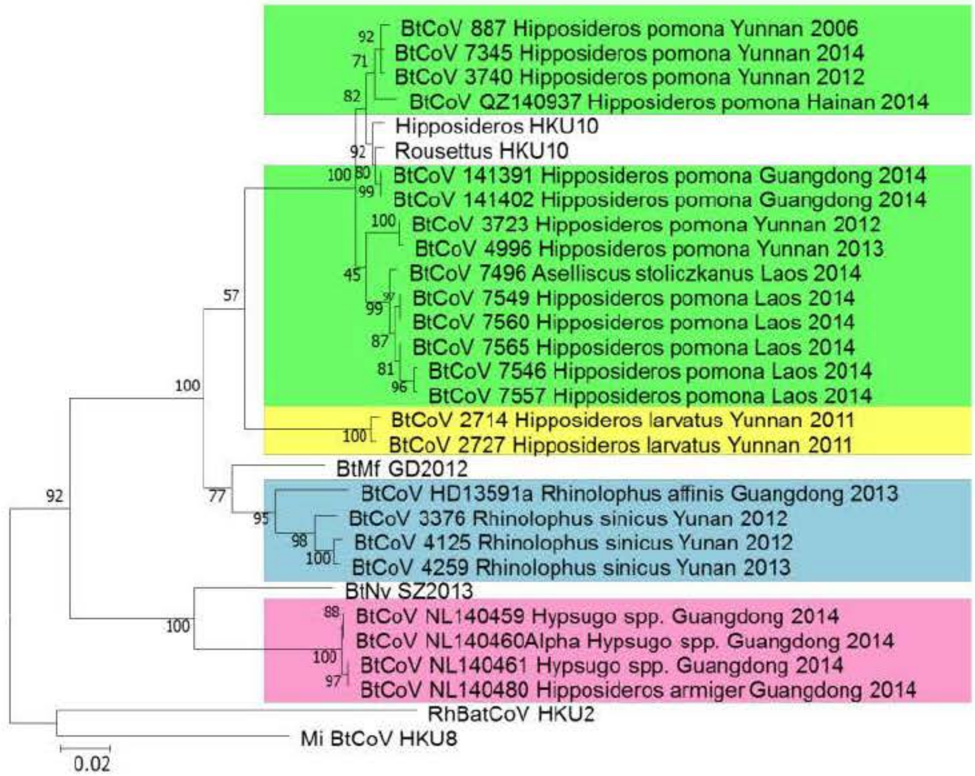


Figure 6. Phylogenetic analysis based on full-length RdRp gene sequence of alpha-CoVs

Genetic Diversity of Receptor-Binding Domain (RBD) of SARS-Like Coronavirus in Chinese Bats

RBD sequences from 37 newly identified SL-CoV from various horseshoe bat species and *Hipposideros* bat species in Yunnan, Guangdong, Guangxi, Hubei and Hunan provinces were amplified and sequenced in Year 3. Phylogenetic analysis revealed that SL-CoV circulating in bat populations in China are highly diverse in the RBD region (*Fi.g 7*). Some strains possessed an RBD sequence distinct from all currently known bat SL-CoVs and formed a new cluster in the phylogenetic tree. However, except for a few strains from Yunnan, most of these SL-CoVs contained nucleotide deletions and were relatively distant to SARS-CoV in the RBD region. These findings suggest that the S gene of SL-CoVs in Chinese bats is even more genetically diverse than expected.

The genomic characterization of SL-CoVs in Year 3 was focused on *Rhinolophus sinicus* in Yunnan, our plan for Year 4 is to obtain complete S gene, RdRp gene or full-length genome sequences of more SL-CoVs from a broader range of bat species identified all over China and conduct a more comprehensive study of the evolution of SL-CoVs in bats.

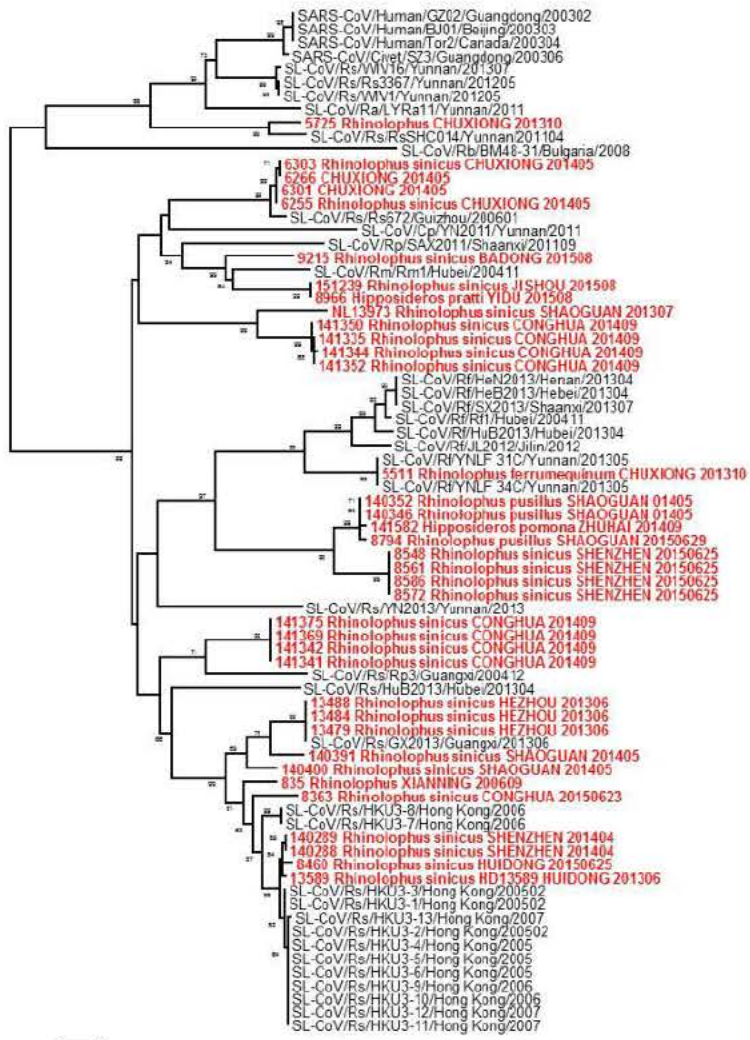


Figure 7. Phylogenetic analysis of the RBD region of the S gene of bat SL-CoVs detected in China (newly identified sequences were marked in red).

Bat Coronavirus Host-virus Phylogeography in China

To analyze the extent to which different bat species and genera are host to similar bat-CoVs, we reconstructed viral phylogenetic relationships and mapped host-species associations onto these phylogenies. Our dataset includes all CoV RdRp sequences isolated from bat specimens collected by our team from 2008-2015 (Alpha-CoVs: n = 491 – Beta-CoVs: n = 326), including those collected under prior NIAID funding (1 R01 AI079231), and funding from Chinese Federal Agencies. All Chinese bat CoV RdRp sequences available in GenBank were also added to our dataset (Alpha-CoVs: n = 226 – Beta-CoVs: n = 206). Phylogenetic trees were reconstructed for Alpha- and Beta-CoVs separately using Bayesian inference and Maximum Likelihood (ML) approaches. RAxML was used to perform ML analysis and Bayesian analyses were performed with MrBayes 3.2.6.

Beta-CoV sequences clustered into four main genetic lineages: B (SARS-CoV and SARS-like CoVs), C (MERS-CoV), D and a potential new lineage related to lineage B (*Fig. 8*). An important phylogenetic structure is observed within lineages C and D. Alpha-CoV sequences clustered into numerous closely related and less-differentiated lineages (*Fig. 9*).

We observed significant CoV lineage sharing among bat genera in our phylogenetic trees. Importantly SARS-like CoVs (SL-CoVs in lineage B) have been detected in Hipposideridae bats in addition to Rhinolophidae bats which were thought to be the putative natural host taxa of SL-CoV (*Fig. 8*). We found additional bat genera that also hosted CoVs in this clade (*Fig. 8*), expanding potential host targets for novel SL-CoV discovery. CoVs closely related to Bat coronavirus HKU9 (lineage D), which were thought to be specific to pteropodid bats, have also been detected in hipposiderid and vespertilionid bats (*Fig. 8*). Important lineage sharing across several bat families has also been observed among most Alpha-CoV lineages (*Fig. 9*). We used host DNA barcoding to confirm these findings - host mitochondrial sequences were generated to confirm the host species identity for most samples.

These results indicate a larger host range, weaker host specificity and higher frequency of cross-genera transmission for most bat CoV lineages than previously thought. These findings will have important implication in our understanding of bat CoV emergence and spillover risk in China. In Year 4 we will expand these analyses to include more explicit co-evolutionary analyses to identify the frequency and timing of host switching events for each major clade.

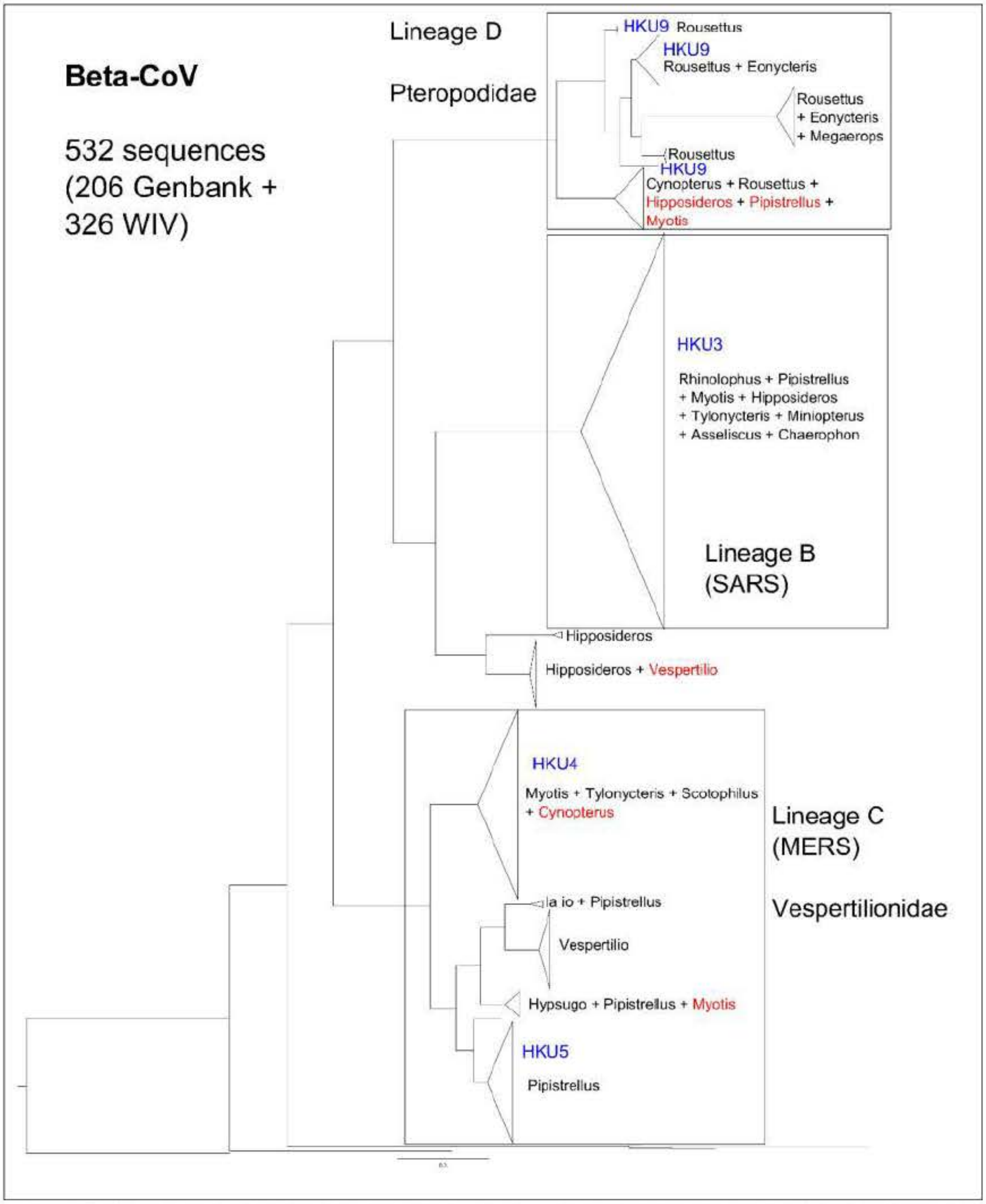


Figure 8. Maximum Likelihood tree of partial RdRp gene sequences of Beta-CoVs. Bat host genera are indicated along each lineage. Bat genera listed in red correspond to minor and potential new bat hosts and may represent cross-genera/family transmission events.

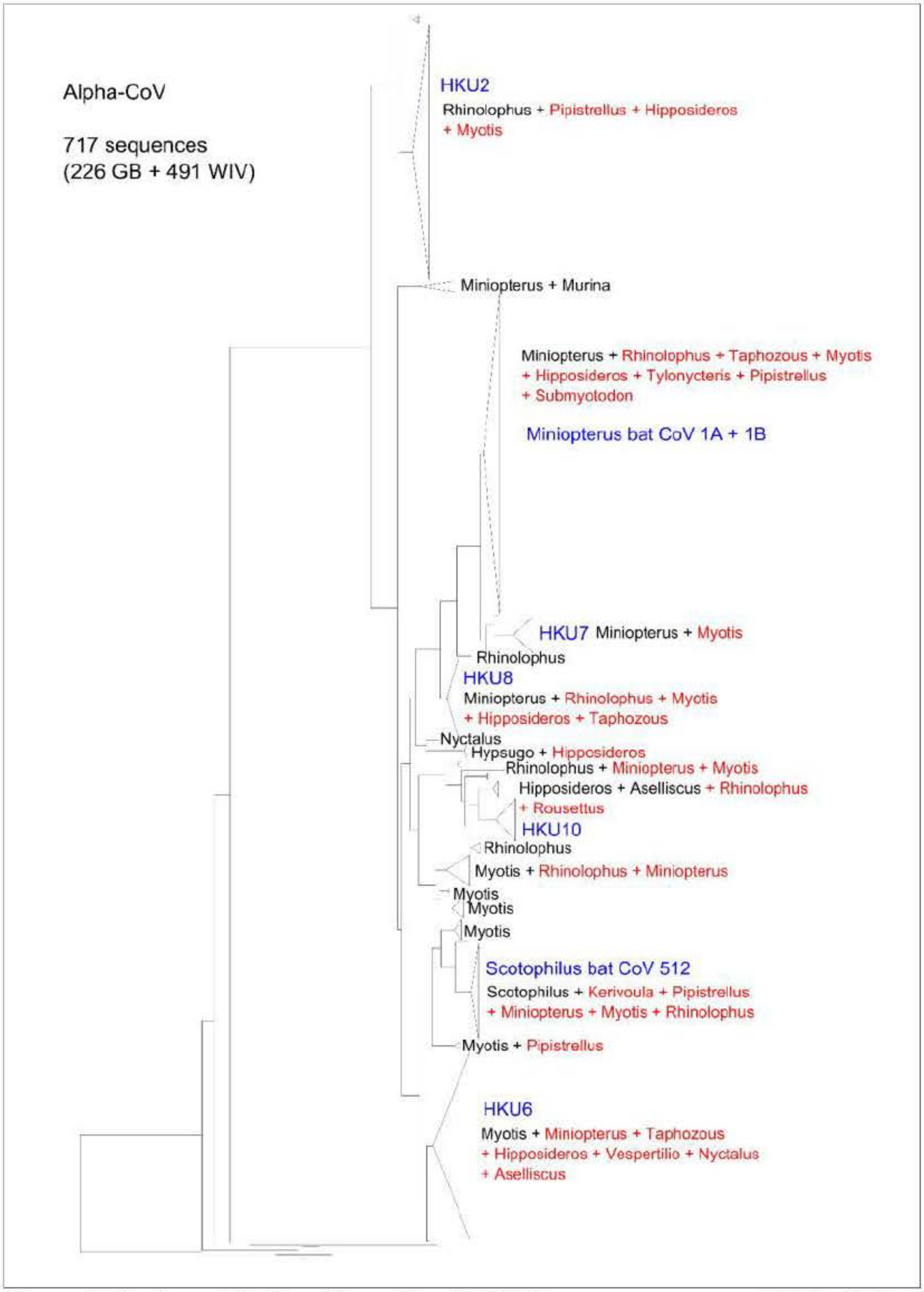


Figure 9. Maximum Likelihood tree of partial RdRp gene sequences of Alpha-CoVs. Bat host genera are indicated along each lineage. Bat genera listed in red correspond to minor and potential new bat hosts and may represent cross-genera/family transmission events.

Global analysis of bat viral sharing to identify key host species

We curated and analyzed a global dataset of bat host–virus associations to better understand the frequency, and connectivity of viral sharing among bats. We also used this to examine the importance of cave-roosting bats species in harboring and sharing viruses with non cave-roosting species, and to identify specific hosts that are central in the network (*Fig. 10*). Cave roosting bat species are host to most CoVs found in bats (orange). We identified global patterns of viral coinfection based on the number of connections between each virus in the network (Fig. 10). We will expand this approach to our China-CoV specific field data in Year 4.

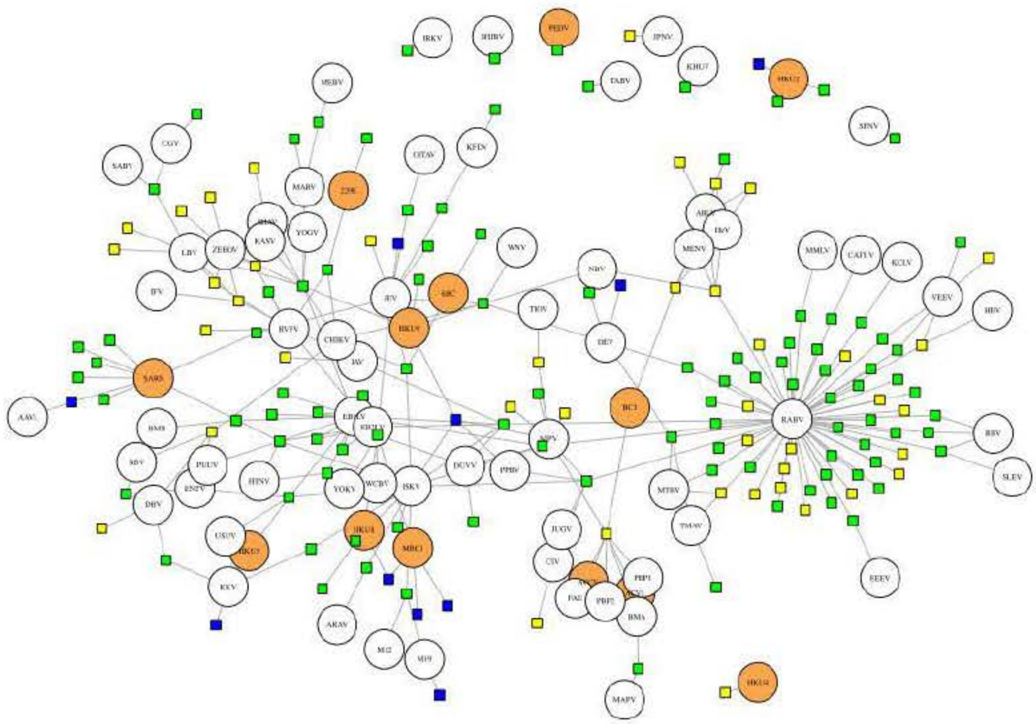


Figure 10. An analysis of global bat virus sharing using data from the published literature combined with field data. Network analysis includes 152 bat host species and 80 ICTV recognized viral species, with 273 host-viral associations. Unique viruses are represented in circles with known CoVs shown in orange, and each square represents a unique bat species. Green squares = facultative cave-roosting bat species; Blue squares = obligate cave-roosting species; Yellow squares = non cave-roosting species. Viruses are linked in the network based on host species that have been observed harboring the same virus – as detected using PCR or viral isolation.

Specific Aim 3: Testing predictions of CoV inter-species transmission

In Year 3 we established an effective and economic reverse genetics system for bat SL-CoV which can be applied to efficiently rescue SL-CoVs that are difficult to culture. This can be used to explore the functions of newly identified SL-CoV genes, as well as to assess pathogenesis of novel bat SL-CoVs. Using this system, we demonstrated that the unique ORFx in WIV1 and WIV16 is a functional gene involving modulation of the host immune response but not essential for *in vitro* viral replication (Zeng et al, 2016, J Virol).

Identification of Three Novel SL-CoVs with Potential for Direct Transmission to Humans
In Y2, we conducted full-length genome sequencing of 11 novel SL-CoVs detected in a single bat habitat in Yunnan province, which included strains highly similar to human/civet SARS-CoV in the most variable genes (N-terminal domain and RBD in the S gene, ORF8 and ORF3) (under revision). Based on recombination analysis, we hypothesized that the direct progenitor of the pandemic SARS-CoV may originated from this location after sequential recombination events at multiple genomic positions.

Among the 11 newly identified SL-CoVs, three different strains namely Rs4874, Rs7327 and Rs4231 contained no deletions in the RBD region but their RBD sequences varied from each other. Rs4874 has an S gene almost identical to that of WIV16. Rs7327's S protein varies from that of WIV1 and WIV16 at three aa residues in the receptor-binding motif, including one contact residue (aa 484) with human ACE2. Rs4231 shares similar NTD sequence with WIV1 and WIV16, but has a distinct RBD sequence. In Year 3, we successfully isolated Rs4874 from the single fecal sample. Using the reverse genetic system we previously developed, we constructed two chimeric viruses with the WIV1 backbone replaced with the S gene of Rs7327 and Rs4231, respectively. Vero E6 cells were respectively infected with Rs4874, WIV1-Rs4231S and WIV1-Rs7327S, and efficient virus replication was detected by immunofluorescence assay in all infections. To assess the usage of human ACE2 by the three novel SL-CoVs, we conducted virus infectivity studies using HeLa cells with or without the expression of human ACE2. All viruses replicated efficiently in the human ACE2-expressing cells. The results were further confirmed by quantification of viral RNA using real-time RT-PCR (*Fig.11*).

These finding suggests that diverse variants of SL-CoV S protein without deletions in their RBD are able to use human ACE2 as receptor for cell entry. Diverse SL-CoVs capable of direct transmission to humans are circulating in bats in southwestern China, which represents a potential risk of emergence given the opportunity to spillover to other animals and/or human populations.

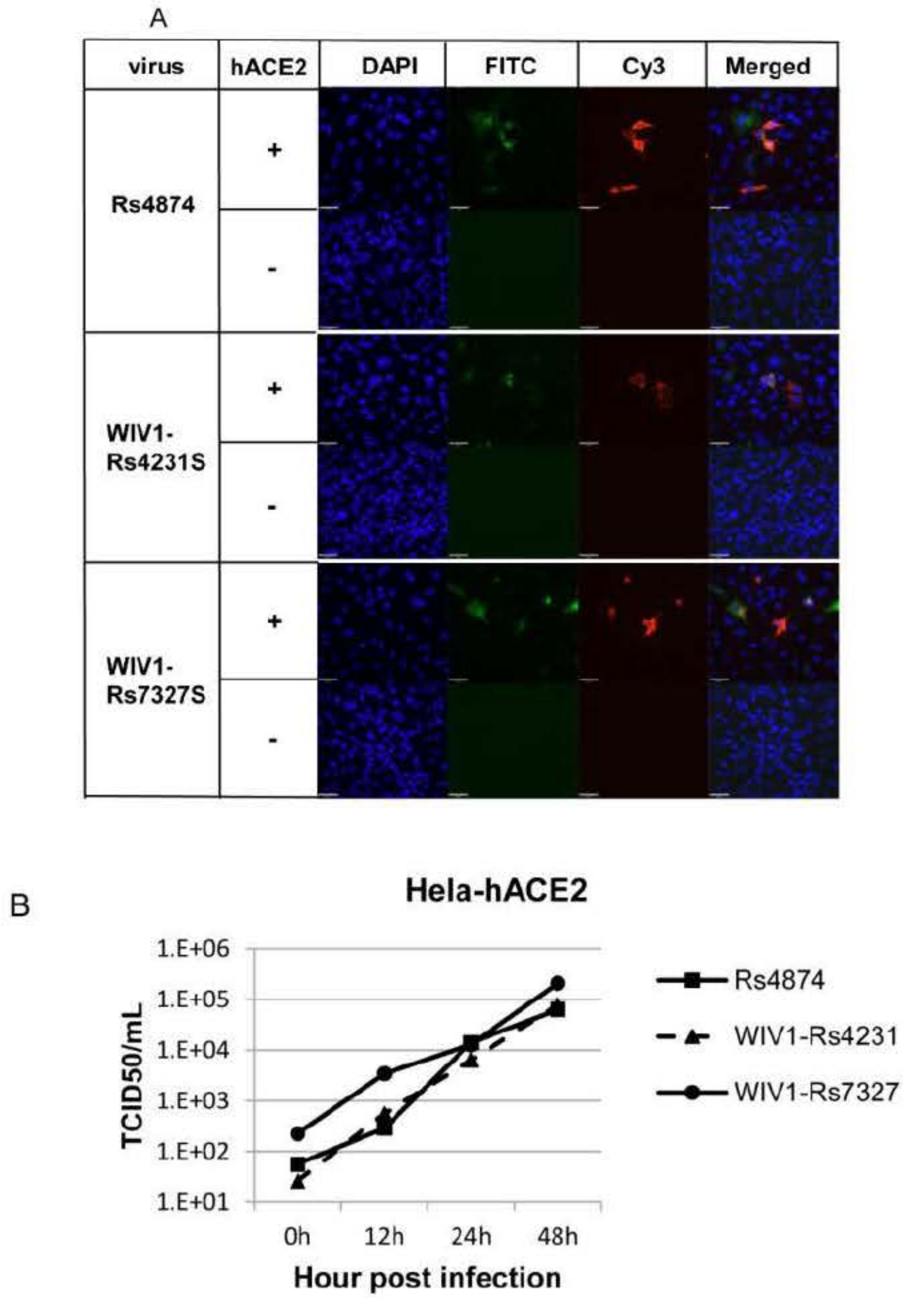


Figure 11. Analysis of receptor usage by immunofluorescence assay (A) and real-time PCR (B).

<u>Additional Year 3 items for Specific Aim 3:</u>

- The full-length infectious cDNA clone of MERS-CoV has been successfully constructed. The full-length S gene of 12 different novel bat MERS-related coronaviruses have been amplified and cloned into the T-vectors. In Y4, we aim to use the reverse genetic method, and construct chimeric viruses with the backbone of MERS-CoV and the S genes from

diverse newly identified bat MERS-related coronaviruses, to examine the pathogenicity of bat MERS-related coronaviruses on cell and animal levels.

- Establishment of animal infection models for bat SL-CoV and MERS-related CoV: Mice with human ACE2 have been imported to China and have been bred for one generation in Wuhan Institute of Virology. Transgenic mice that express human DPP4 have also been constructed and are being bred. The animal infection experiments are planned to be conducted in following years to study the pathogenicity of diverse SL-CoVs and MERS-related CoV that we identified in Chinese bats.

Specific Goal Not Meet

- Observations and animal sampling at wildlife markets were not done in Year 3 because the stricter law enforcement and subsequent cautiousness of traders make it difficult to access to wild animal in markets. Instead, we piloted the wild animal farm survey and will be focusing on it in Year 4, with evidence from pre-investigations that shows most wild animal farms serve as transit points during the wildlife trade.
- The passive hospital surveillance has been piloted in Year 3 and will continue in Year 4 to collect and test samples for SL-CoV and other viral families
- Cophylogenetic analyses of bat host and CoV phylogenies to assess patterns of evolutionary congruence and frequency of cross-species transmission to be continued in Year 4
- Animal infection experiments of SL-CoVs and MERS-related CoV were not done in Year 3, as this is planned as part of work in Year 4.

Significant Oral Presentations

1. Daszak P. Plenary talk, One Health-EcoHealth Congress, Melbourne, Dec. 2016
2. Daszak P. 2nd annual Global Pandemic Policy Summit, Scowcroft Ctr, Texas A&M Univ.
3. Daszak P. Global Health Security Agenda side event, UN World Humanitarian Summit: FAO/WHO/USAID/Global He@lth 2030 Innovation Task Force; Istanbul, Turkey.
4. Daszak P. Symposium at École du Val-de-Grâce, Paris
5. Daszak P. Plenary, Institute of Zoology symposium on Bushmeat and disease risks, London.
6. Daszak P. Duke University Provost's Forum on Conservation and Health
7. Olival KJ. The 17th International Bat Research Conference "Assessing the Risk of Disease Emergence from Bat Hunting: Overview and Implications for Risk Mitigation". Durban, South Africa, 2016
8. Daszak P. American Public Health Association Annual Meeting 2016 "Preliminary Results from An Innovative One Health Behavioral Surveillance System". Denver, 2016

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

We presented this work to the chief physicians, nurses, and directors from county-level clinics in Guangdong and Yunnan provinces during the implementation of Integrated Biological Behavioral Surveillance in Chuxiong and Guangzhou. All the research staff were trained and re-trained for the biosafety and PPE use for human biological sampling.

11 graduate students from School of Public Health of Wuhan University and Wuhan Institute of Virology of CAS were trained for laboratory and field biosafety and PPE use, behavioral data collection methodologies and technologies, and data analysis.

Research Technician Dr. Guangjian Zhu was invited by the Institute of Pathogen Biology, Chinese Academy of Medical Science & Peking Union Medical College to provide training to 10 field team members regarding biosafety and PPE use, bats and rodents sampling.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Non-Compliant	(b) (4) [redacted]
Complete	Zeng LP, Gao YT, Ge XY, Zhang Q, Peng C, Yang XL, Tan B, Chen J, Chmura AA, Daszak P, Shi ZL. Bat Severe Acute Respiratory Syndrome-Like Coronavirus WIV1 Encodes an Extra Accessory Protein, ORFX, Involved in Modulation of the Host Immune Response. Journal of virology. 2016 July 15;90(14):6573-82. PubMed PMID: 27170748; PubMed Central PMCID: PMC4936131.
Complete	Olival KJ, Willoughby AR. Prioritizing the 'Dormant' Flaviviruses. EcoHealth. 2017 March;14(1):1-2. PubMed PMID: 28194584; PubMed Central PMCID: PMC5386397.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
(b) (6) [redacted]	Y	DASZAK, PETER	BS,PHD	PD/PI	[redacted]	[redacted]	(b) (4), (b) (6) [redacted]			NA
	N	KE, CHANGWEN	PHD	Co-Investigator	[redacted]	[redacted]	[redacted]	Center for Disease Control and Prevention of Guangdong Province	CHINA	NA
(b) (6) [redacted]	N	Ross, Noam Martin	PhD	Co-Investigator	[redacted]	[redacted]	[redacted]			NA
	N	SHI, ZHENGLI	PhD	Co-Investigator	[redacted]	[redacted]	[redacted]	Wuhan Institute of Virology	CHINA	NA
	N	OLIVAL, KEVIN J	PHD	Co-Investigator	[redacted]	[redacted]	[redacted]			NA
	N	ZHANG, YUNZHI	PHD	Co-Investigator	[redacted]	[redacted]	[redacted]	Yunnan Provincial Institute of Endemic Diseases Control & Prevention	CHINA	NA
	N	ZHU, GUANGJIAN	PHD	Co-Investigator	[redacted]	[redacted]	[redacted]	East China Normal University	CHINA	NA
	N	GE, XINGYI	PHD	Co-Investigator	[redacted]	[redacted]	[redacted]	Wuhan Institute of Virology	CHINA	NA
	N	EPSTEIN, JONATHAN H	MPH,DVM,BA,PHD	Co-Investigator	[redacted]	[redacted]	[redacted]			NA
	N	CHMURA, ALEKSEI A	BS	Non-Student Research Assistant	[redacted]	[redacted]	[redacted]			NA
	N	ZHANG, SHUYI	PHD	Co-Investigator	[redacted]	[redacted]	[redacted]	East China Normal University	CHINA	NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Dollar Amount	Country
213239	CHINA

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

Yes

Is the research exempt from Federal regulations?

No

Does this project involve a clinical trial?

No

G.4.b Inclusion Enrollment Data

Report Attached: Understanding the Risk of Bat Coronavirus Emergence-PROTOCOL-001

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

No

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

No

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional	Address

		District	
Primary: EcoHealth Alliance, Inc.	077090066	NY-010	460 West 34th Street 17th Floor New York NY 100012317
Wuhan Institute of Virology	529027474		Xiao Hong Shan, No. 44 Wuchang District Wuhan
Wuhan University School of Public Health	549376772	00-000	115 Donghu Road Wuhan nullnull

G.9 FOREIGN COMPONENT

Organization Name: Wuhan Institute of Virology
Country: CHINA
Description of Foreign Component:
Principal Laboratory for all Research in China as per section G8 (above) and detailed in our Specific Aims

Organization Name: Wuhan School of Public Health
Country: CHINA
Description of Foreign Component:
Principal Coordinating Team for all project field work as per section G8 (above) and detailed in our Specific Aims

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

Inclusion Enrollment Report

Inclusion Data Record (IDR) #: 166195

Using an Existing Dataset or Resource: No

Delayed Onset Study ?: No

Clinical Trial: No

Enrollment Location: Foreign

NIH Defined Phase III Clinical Trial: No

Study Title: Understanding the Risk of Bat Coronavirus Emergence-PROTOCOL-001

Planned Enrollment

Planned Enrollment Total: 2,460

NOTE: Planned enrollment data exists in the previous format; the PD/PI did not enter the planned enrollment information in the modified format and was not required to do so. Only the total can be provided.

Cumulative Enrollment

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	0	0	0	0	0	0	0	0	**0**
Asian	708	459	0	0	0	0	0	0	0	**1167**
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	**0**
Black or African American	0	0	0	0	0	0	0	0	0	**0**
White	0	0	0	0	0	0	0	0	0	**0**
More than One Race	0	0	0	0	0	0	0	0	0	**0**
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	**0**
Total	**708**	**459**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1167**

RESEARCH **Federal Award Date:** 06/18/2018

Department of Health and Human Services
National Institutes of Health

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI110964-05
FAIN: R01AI110964

Principal Investigator(s):
PETER DASZAK, PHD

Project Title: Understanding the Risk of Bat Coronavirus Emergence

Aleksei Chmura
President
460 West 34th Street
17th Floor
New York, NY 100012317

Award e-mailed to: [redacted] (b) (6)

Period Of Performance:
Budget Period: 06/01/2018 – 05/31/2019
Project Period: 06/01/2014 – 05/31/2019

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $581,646 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to ECOHEALTH ALLIANCE, INC. in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI110964. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Tseday G Girma
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI110964-05

Award Calculation (U.S. Dollars)

Salaries and Wages	$167,708
Fringe Benefits	$54,168
Personnel Costs (Subtotal)	$221,876
Materials & Supplies	$3,500
Travel	$35,918
Other	$9,400
Subawards/Consortium/Contractual Costs	$191,576

Federal Direct Costs	$462,270
Federal F&A Costs	$119,376
Approved Budget	$581,646
Total Amount of Federal Funds Obligated (Federal Share)	$581,646
TOTAL FEDERAL AWARD AMOUNT	$581,646

AMOUNT OF THIS ACTION (FEDERAL SHARE)	$581,646

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
5	$581,646	$581,646

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1311726494A1
Document Number:	RAI110964A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2018

IC	CAN	2018
AI	8472350	$581,646

NIH Administrative Data:
PCC: M51C / **OC**: 414E / **Released**: [redacted] (b) (6) 06/15/2018
Award Processed: 06/18/2018 12:02:35 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI110964-05

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI110964-05

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI110964. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award represents the final year of the competitive segment for this grant. See the NIH Grants Policy Statement Section 8.6 Closeout for complete closeout requirements at: http://grants.nih.gov/grants/policy/policy.htm#gps.

A final expenditure Federal Financial Report (FFR) (SF 425) must be submitted through the eRA Commons (Commons) within 120 days of the period of performance end date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports, http://grants.nih.gov/grants/policy/policy.htm#gps, for additional information on this submission requirement. The final FFR must indicate the exact balance of unobligated funds and may not reflect any unliquidated obligations. There must be no discrepancies between the final FFR expenditure data and the Payment Management System's (PMS) quarterly cash transaction data. A final quarterly federal cash transaction report is not required for awards in PMS B subaccounts (i.e., awards to foreign entities and to Federal agencies). NIH will close the awards using the last recorded cash drawdown level in PMS for awards that do not require a final FFR on expenditures or quarterly federal cash transaction reporting. It is important to note that for financial closeout, if a grantee fails to submit a required final expenditure FFR, NIH will close the grant using the last recorded cash drawdown level. If the grantee submits a final expenditure FFR but does not reconcile any discrepancies between expenditures reported on the final expenditure FFR and the last cash report to PMS, NIH will close the award at the lower amount. This could be considered a debt or result in disallowed costs.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted within 120 days of the expiration date. The HHS 568 form may be downloaded at: http://grants.nih.gov/grants/forms.htm. This paragraph does not apply to Training grants, Fellowships, and certain other programs—i.e., activity codes C06, D42, D43, D71, DP7, G07, G08, G11, K12, K16, K30, P09, P40, P41, P51, R13, R25, R28, R30, R90, RL5, RL9, S10, S14, S15, U13, U14, U41, U42, U45, UC6, UC7, UR2, X01, X02.

Unless an application for competitive renewal is submitted, a Final Research Performance Progress Report (Final RPPR) must also be submitted within 120 days of the period of performance end date. If a competitive renewal application is submitted prior to that date, then an Interim RPPR must be submitted by that date as well. Instructions for preparing an Interim or Final RPPR are at: https://grants.nih.gov/grants/rppr/rppr_instruction_guide.pdf. Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the Interim or Final RPPR. *Note that data reported within Section I of the Interim and Final RPPR forms will be made public and should be written for a lay person audience.*

NIH strongly encourages electronic submission of the final invention statement through the Closeout feature in the Commons, but will accept an email or hard copy submission as indicated below.

Email: The final invention statement may be e-mailed as PDF attachments to: NIHCloseoutCenter@mail.nih.gov.

Hard copy: Paper submissions of the final invention statement may be faxed to the NIH Division of Central Grants Processing, Grants Closeout Center, at 301-480-2304, or mailed to:

National Institutes of Health
Office of Extramural Research
Division of Central Grants Processing
Grants Closeout Center
6705 Rockledge Drive
Suite 5016, MSC 7986
Bethesda, MD 20892-7986 (for regular or U.S. Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express deliveries only)

NOTE: If this is the final year of a competitive segment due to the transfer of the grant to another institution, then a Final RPPR is not required. However, a final expenditure FFR is required and should be submitted electronically as noted above. If not already submitted, the Final Invention Statement is required and should be sent directly to the assigned Grants Management Specialist.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI110964-05

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

If any experiments proposed in this award result in a virus with enhanced growth by more than 1 log compared to wild type strains, you must notify your NIAID Program Officer and Grants Management Specialist immediately. Further research involving the resulting virus(es) may require review by the Department of Health and Human Services in accordance with the Framework for Guiding Funding Decisions about Proposed Research Involving Enhanced Potential Pandemic Pathogens (https://www.phe.gov/s3/dualuse/Documents/P3CO.pdf).

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The Research Performance Progress Report (RPPR), Section G.9 (Foreign component), includes reporting requirements for all research performed outside of the United States. Research conducted at the following site(s) must be reported in your RPPR:

San Pya Clinic, BURMA
Institut Pasteur du Cambodge, CAMBODIA
Primate Research Center at Bogor Agricultural University, INDONESIA
Conservation Medicine, Ltd, MALAYSIA
King Chulalongkorn Memorial Hospital, THAILAND
Hanoi Agricultural University, VIETNAM
National Animal Health Laboratory, LAOS

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This Notice of Award (NoA) includes collaboration with **Wuhan University School of Public Health, CHINA.**

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This Notice of Award (NoA) includes funds for activity with **Wuhan Institute of Virology, CHINA.**

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This Notice of Award (NoA) includes funds for activity with **East China Normal University.**

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This award may include collaborations with and/or between foreign organizations. Please be advised that short term travel visa expenses are an allowable expense on this grant, if justified as critical and necessary for the conduct of the project.

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This award is subject to the Clinical Terms of Award included in Monitoring of Clinical Trials and Studies - NIAID (see NIH Guide for Grants and Contracts, July 8, 2002, NOT AI-02-032). These terms and conditions are hereby incorporated by reference, and can be accessed via the following World Wide Web address: https://www.niaid.nih.gov/grants-contracts/niaid-clinical-terms-award All submissions required by the NIAID Clinical Terms of Award must be forwarded electronically or by mail to the responsible NIAID Program Official identified on this Notice of Award.

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Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

> Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.
>
> If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:
>
> - o A list of the new and/or additional Agent(s) that will be studied;
> - o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
> - o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Adam Graham
Email: [redacted] (b) (6) **Phone**: [redacted] (b) (6) **Fax**: 301-493-0597

Program Official: Erik J. Stemmy
Email: [redacted] (b) (6) **Phone**: [redacted] (b) (6)

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI110964-05

INSTITUTION: ECOHEALTH ALLIANCE, INC.

Budget	Year 5
Salaries and Wages	$167,708
Fringe Benefits	$54,168
Personnel Costs (Subtotal)	$221,876
Materials & Supplies	$3,500
Travel	$35,918
Other	$9,400
Subawards/Consortium/Contractual Costs	$191,576
TOTAL FEDERAL DC	$462,270
TOTAL FEDERAL F&A	$119,376
TOTAL COST	$581,646

Facilities and Administrative Costs	Year 5
F&A Cost Rate 1	44.1%
F&A Cost Base 1	$270,694

F&A Costs 1	$119,376

A. COVER PAGE

Project Title: Understanding the Risk of Bat Coronavirus Emergence	
Grant Number: 5R01AI110964-05	**Project/Grant Period:** 06/01/2014 - 05/31/2019
Reporting Period: 06/01/2017 - 05/31/2018	**Requested Budget Period:** 06/01/2018 - 05/31/2019
Report Term Frequency: Annual	**Date Submitted:** 09/16/2020
Program Director/Principal Investigator Information: PETER DASZAK , PHD BS **Phone number:** [redacted] (b) (6) **Email:** [redacted] (b) (6)	**Recipient Organization:** ECOHEALTH ALLIANCE, INC. ECOHEALTH ALLIANCE, INC. 460 W 34TH ST 17TH FLOOR NEW YORK, NY 100012320 **DUNS:** 077090066 **EIN:** 1311726494A1 **RECIPIENT ID:** NIAID Coronavirus
Change of Contact PD/PI: N/A	
Administrative Official: ALEKSEI CHMURA 460 W 34th St., 17th Floor New York, NY 10001 **Phone number:** [redacted] (b) (6) **Email:** [redacted] (b) (6)	**Signing Official:** ALEKSEI CHMURA 460 W 34th St., 17th Floor New York, NY 10001 **Phone number:** [redacted] (b) (6) **Email:** [redacted] (b) (6)
Human Subjects: Yes HS Exempt: No Exemption Number: Phase III Clinical Trial:	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Zoonotic coronaviruses are a significant threat to global health, as demonstrated with the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, and the recent emergence Middle East Respiratory Syndrome (MERS-CoV). The wildlife reservoirs of SARS-CoV were identified by our group as bat species, and since then hundreds of novel bat-CoVs have been discovered (including >260 by our group). These, and other wildlife species, are hunted, traded, butchered and consumed across Asia, creating a largescale human-wildlife interface, and high risk of future emergence of novel CoVs.
To understand the risk of zoonotic CoV emergence, we propose to examine 1) the transmission dynamics of bat-CoVs across the human-wildlife interface, and 2) how this process is affected by CoV evolutionary potential, and how it might force CoV evolution. We will assess the nature and frequency of contact among animals and people in two critical human-animal interfaces: live animal markets in China and people who are highly exposed to bats in rural China. In the markets we hypothesize that viral emergence may be accelerated by heightened mixing of host species leading to viral evolution, and high potential for contact with humans. In this study, we propose three specific aims and will screen free ranging and captive bats in China for known and novel coronaviruses; screen people who have high occupational exposure to bats and other wildlife; and examine the genetics and receptor binding properties of novel bat-CoVs we have already identified and those we will discover. We will then use ecological and evolutionary analyses and predictive mathematical models to examine the risk of future bat-CoV spillover to humans. This work will follow 3 specific aims:

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces. We will examine if: 1) wildlife markets in China provide enhanced capacity for bat-CoVs to infect other hosts, either via evolutionary adaptation or recombination; 2) the import of animals from throughout Southeast Asia introduces a higher genetic diversity of mammalian CoVs in market systems compared to within intact ecosystems of China and Southeast Asia; We will interview people about the nature and frequency of contact with bats and other wildlife; collect blood samples from people highly exposed to wildlife; and collect a full range of clinical samples from bats and other mammals in the wild and in wetmarkets; and screen these for CoVs using serological and molecular assays.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk. We propose two competing hypotheses: 1) CoV host-range in bats and other mammals is limited by the
phylogenetic relatedness of bats and evolutionary conservation of CoV receptors; 2) CoV host-range is limited by geographic and ecological opportunity for contact between species so that the wildlife trade disrupts the 'natural' co-phylogeny, facilitates spillover and promotes viral evolution. We will develop CoV phylogenies from sequence data collected previously by our group, and in the proposed study, as well as from Genbank. We will examine co-evolutionary congruence of bat-CoVs and their hosts using both functional (receptor) and neutral genes. We will predict host-range in unsampled species using a generalizable model of host and viral ecological and phylogenetic traits to explain patterns of viral sharing between species. We will test for positive selection in market vs. wild-sampled viruses, and use data to parameterize mathematical models that predict CoV evolutionary and transmission dynamics. We will then examine scenarios of how CoVs with different transmissibility would likely emerge in wildlife markets.

Specific Aim 3: Testing predictions of CoV inter-species transmission. We will test our models of host range (i.e. emergence potential) experimentally using reverse genetics, pseudovirus and receptor binding assays, and virus infection experiments in cell culture and humanized mice. With bat-CoVs that we've isolated or sequenced, and using live virus or pseudovirus infection in cells of different origin or expressing different receptor molecules, we will assess potential for each isolated virus and those with receptor binding site sequence, to spill over. We will do this by sequencing the spike (or other receptor binding/fusion) protein genes from all our bat-CoVs, creating mutants to identify how significantly each would need to evolve to use ACE2, CD26/DPP4 (MERS-CoV receptor) or other potential CoV receptors. We will then use receptor-mutant pseudovirus binding assays, in vitro studies in bat, primate, human and other species' cell lines, and with humanized mice where particularly interesting viruses are identified phylogenetically, or isolated. These tests will provide public health-relevant data, and also iteratively improve our predictive model to better target bat species and CoVs during our field studies to obtain bat-CoV strains of the greatest interest for understanding the mechanisms of cross-species transmission.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Year 4 NIAID CoV Report_Final for eRA Commons.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: Year 4 NIAID CoV Training and Prof Devlp.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

1.Conference and University Lectures: PI Daszak, and Co-investigators Shi, Epstein, Olival, and Zhang gave invited University and Conference lectures including Harvard Univ. Columbia Univ., Tufts Univ., Mt. Sinai, the 2nd International Symposium on Emerging Viral Disease in China, the 2nd International Symposium on the Infectious Diseases of Bats in Colorado, Cell Symposia: Emerging and Re-emerging Viruses 2017 in Virginia, The International Union of Microbiological Societies 2017 National Academy of Sciences in Singapore, 2018 Borneo Quality of Life Conference in Malaysia, 2017 Chemical and Biological Defense Science and Technology (CBD S&T) in California, Prince Mahidol Award Conference in Bangkok, Collaboration for Environmental Evidence Meeting in Paris, US-China NSF Ecology and Evolution of Infectious Disease (EEID) Meeting, and others that included specific discussion of the current project and results.

2.Agency and other briefings: PI Daszak and Co-investigator Shi introduced this project and discussed new opportunities about predicting and preventing zoonoses within National Institute of Allergy and Infectious Disease Office, Defense Advanced Research Projects Agency, National Natural Science Foundation of China, Chinese Center for Disease Control and Prevention, US NASEM Forum on Microbial Threats, Chinese Academy of Sciences, and the Health Working Group at the US Embassy in Beijing.

3.Public outreach: PI Daszak and Co-investigator Shi, Epstein, Olival, have presented this work to the general public in a series of meetings over Year 4 including at Cosmos Club briefings that EcoHealth Alliances hosts in Washington DC, over 10 meetings on the China National Virome Project and the Global Virome Project in China, Europe, Australia, Southeast Asia and Latin America. Co-investigator Olival presented this work at a public event on Disease Transmission and Technologies in New York, co-investigator Ross presented this work at EcoHealth Webinar on wildlife trade network research. Zhu broadly introduced this work to the conservation and ecological research community in China through field training workshops.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Specific Aim 1: Assessment of CoV spillover potential at high risk human-wildlife interfaces.

•To commence an in-depth analysis of data collected from the integrated biological behavioral surveillance from Yunnan, Guangxi, and Guangdong provinces, incorporating questionnaires and serological testing results.
•To initiate lab analysis of human samples collected from the passive hospital surveillance from four hospitals in Yunnan province: 1) Dali College Affiliated Hospital; 2) Dali Prefecture Hospital; 3) Kunming No. 3 People's Hospital, and 4) Chuxiong Prefecture Hospital. The goal will be to identify examples of CoV spillover events in China that may lead to illness.

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk

•To repeat and continue in vivo experiments of SARSr-CoVs with spike variants on hACE-expressing transgenic mice (survival rate, histopathological analysis, etc) to evaluate the risk of cross-species infection of different SARSr-CoVs to humans;
•Continue searching for the receptor of SARSr-CoVs with deletions in the homologous region of SARS-CoV RBD (i.e. Rp3, Rs672), and SARSr-CoVs that are unable to utilize bat ACE2 (e.g. Rs4231).
•Continue the phylogeographic study of bat-CoV with newly collected samples to better understand the geographic distribution and evolution of bat-CoV genetic diversity in south China and SE Asia.

Specific Aim 3: Testing predictions of CoV inter-species transmission.

•Using the full-length infectious cDNA clone of MERS-CoV, chimeric viruses with the spikes of newly identified MERSr-CoVs will be constructed. The pathogenesis of these MERSr-CoVs will be tested on the human DPP4-expressing mouse model that has already been developed and validated in Y4.
•To conduct a population genetics study of Rhinolophus sinicus ACE2s, including the amplification of ACE2 genes from R. sinicus samples of different origin, test of the usage efficiency of R. sinicus ACE2s of different origins by SL-CoVs and kinetics study on the binding of SL-CoV RBD to different R. sinicus ACE2s.
•In collaboration with South China Agrricultural University, gather data on the spatial structure and barn-level mortality records to parameterize our mathematical model of virus spread that incorporates a meta-population structure in individual and use this to fit the model on a training set of farms and validate it on a hold-out set.
•Using the intra-farm transmission model, we will (a) determine the characteristics of a farm that determine the likelihood and size of an outbreak given a spillover event, and (b) determine whether SADS and PEDV outbreaks on farms can be distinguished by differing dynamics, as measured by transmission parameters in our intra-farm transmission model.

Year 4 Report: Understanding the Risk of Bat Coronavirus Emergence

Award Number: R01AI110964-03

Reporting Period: 06/01/2017 – 05/31/2018

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B.2 What was accomplished under these goals?

Summary

The results of the 4th year of our R01 work are detailed below. They include:

- Completed behavioral risk survey questionnaires and biological sample data collection for 1,585 people in Yunnan, Guangxi, and Guangdong provinces.
- Preliminary analysis of behavioral survey responses exploring key risk factors relating to potential viral zoonotic disease spillover in China, indicating notable differences among the respondents in Guangdong, Guangxi, and Yunnan.
- Completed serologic testing of collected human samples for MERS-CoV, SARSr-CoV, HKU9 CoV and HKU10 CoV, showing the serologic evidence of spillover of bat SARS-related CoVs (7 people in Yunnan province) and HKU9 CoV (2 people in Guangxi province).
- Testing of samples from 671 individual bats to identify diverse alpha- and beta-coronaviruses.
- Genetic diversity and genomic characterization of beta-coronaviruses in fruit bats and characterization of the full-length genome sequence of a novel HKU9-related CoV.
- Analysis of host-virus phylogeography for all bat CoV RdRp sequences collected by our group in China from 2008-2015 (Alpha-CoVs: n = 491; Beta-CoVs: n = 326) to identify the geographic areas that are likely sources of origin/diversity for this important group of viruses.
- Identification of two novel MERS-related CoVs that use DPP4 receptor.
- *In vivo* infection of SARSr-CoVs with variants of S protein in human ACE2 (hACE2) expressing mice.
- Identification of a novel bat-origin CoV (swine acute diarrhea syndrome coronavirus, SADS-CoV) causing a multi-farm outbreak of fatal acute diarrhea in piglets in Guangdong (published in *Nature* in April 2018).
- Development of an intra-farm transmission model to understand SADS-CoV spread and help predict and prevent future outbreaks.

Specific Aim 1: Assessment of CoV spillover potential at high-risk human-wildlife interfaces

During Year 4 we completed behavioral risk surveys and biological sample collection from people at selected sites in three provinces in southern China (Guangdong, Guangxi, and Yunnan) and began analyzing the results.

Behavioral Survey

We administered 1,585 surveys in Guangdong, Guangxi, and Yunnan provinces. Questions explored respondent health-seeking behavior, experiences with unusual illnesses, contact with wildlife and livestock, and general background information. Blood samples were collected from respondents and tested for SAS-related CoVs (SARSr-CoVs) and HKU10-CoV using serological assays. Survey data was analyzed by province to examine patterns among respondent characteristics and behavioral risk factors across provinces.

Respondent General Background Information

Of the 1,585 respondents who completed the survey, 420 were from Guangdong, 412 were from Guangxi, and 753 were from Yunnan. More females than males completed the survey in all provinces. The mean age of the overall survey sample was 52 years **(Figs. 1, 2)**.

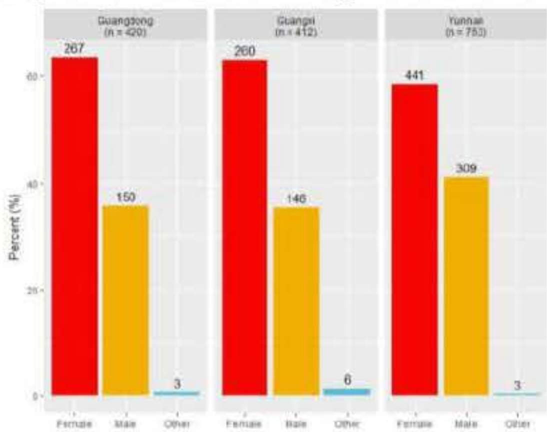


Figure 1: *Gender of respondents*

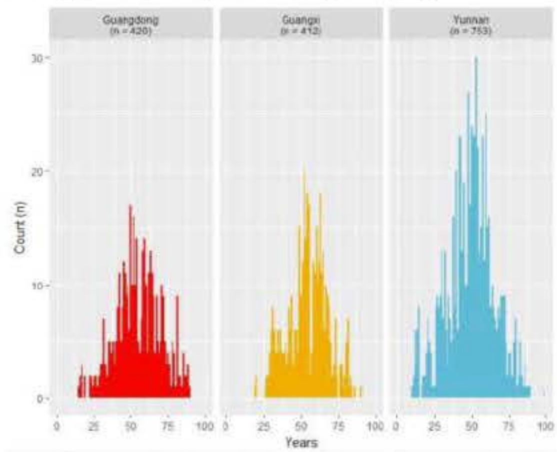


Figure 2: *Age distribution of respondents.*

Across all provinces, most respondents had lived in their respective locales for more than 5 years (96.3%) **(Fig. 3)** and earned less than 10,000 renminbi (RMB) annually (84.6%) **(Fig. 4)**. In 2016, the updated poverty standard in China was 3,000 RMB as defined by Poverty Alleviation Office of State Council. More families in Guangxi (61.8%) lived at or below the poverty level as compared to those in Guangdong (36.9%) and Yunnan (43.3%).

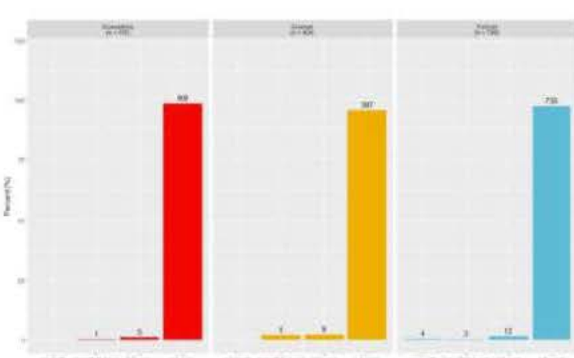


Figure 3: *Duration of residency.*

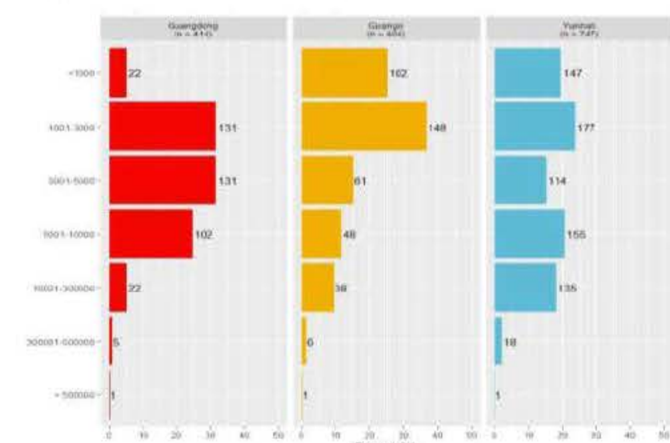


Figure 4: *Family annual per capita income (RMB).*

In Guangdong, Guangxi, and Yunnan, 73.9%, 57.0% and 69.6% of respondents, respectively, had a primary school-level education or less **(Fig. 5)**. Across all provinces the most common livelihood was crop production. In Yunnan, 699 out of 753 (92.8%) individuals from the province identified crop production as a livelihood activity. In comparison, 237 out of 420 (56.4%) individuals from Guangdong, and 260 out of 412 (63.1%) individuals from Guangxi **(Fig. 6)** named crop production as a livelihood in the last year. Respondents, however, where not restricted to defining a single livelihood, many indicated engaging in multiple types of livelihoods.

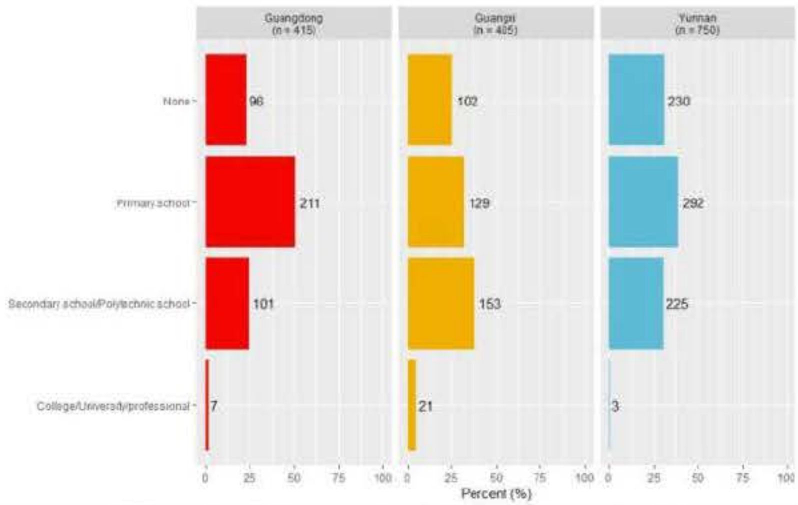


Figure 5: *Highest level of education completed*

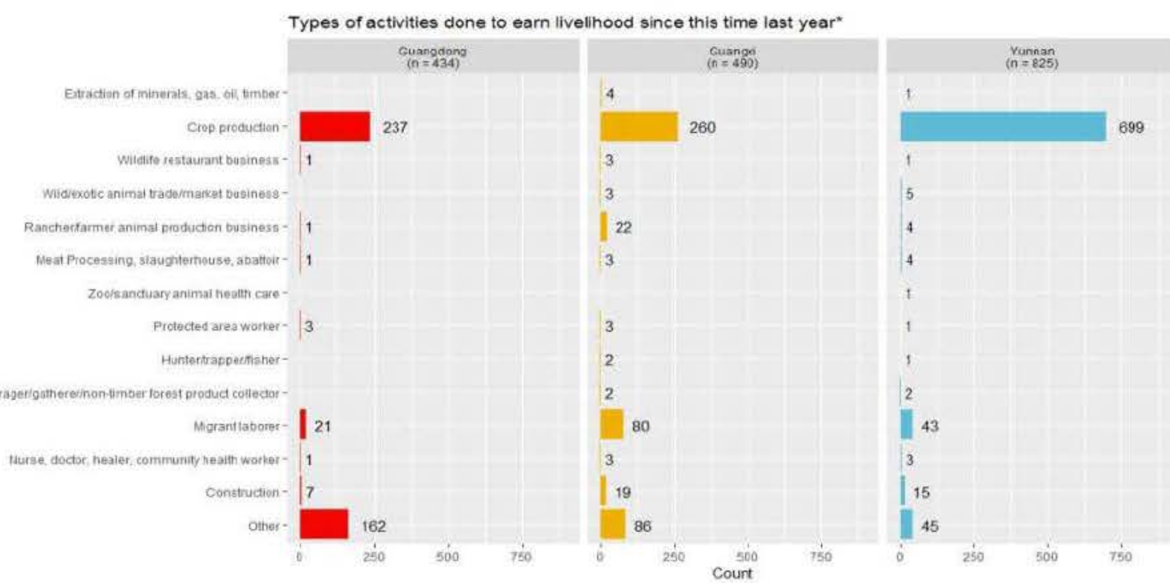


Figure 6: *Types of activities conducted to earn a livelihood since this time last year (above)*

In Guangdong, Guangxi, and Yunnan, 41.7%, 50.7% and 59.6% of respondents, respectively, indicated that they traveled outside of their village town or city in the past year. Among those who traveled, the average number of trips was 5 in Guangdong and Guangxi, and 6 in Yunnan. The average distance traveled by respondents in Guangdong and Yunnan were 113 Km and 118 Km, respectively, compared to 66 Km by respondents in Guangxi.

Health-Seeking Behavior and Experiences with Unusual Illnesses

When asked where they usually get treatment for illness or infection, the top 3 responses across all provinces in aggregate were hospitals, clinics, and pharmacies/dispensaries in descending order **(Fig. 7)**. However, within Yunnan, most respondents went to hospitals, followed by pharmacies, then clinics.

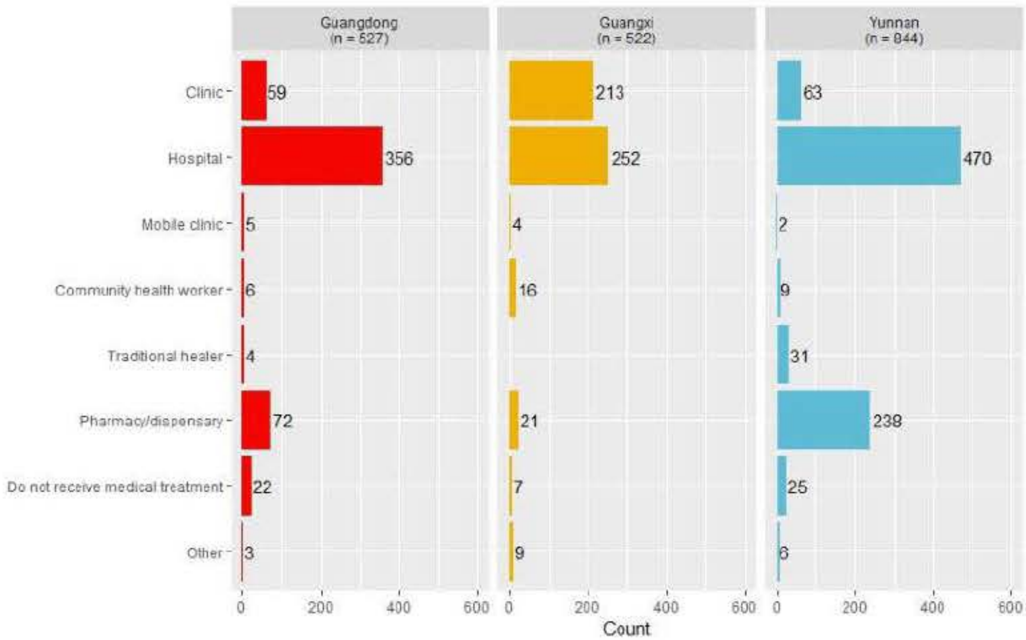
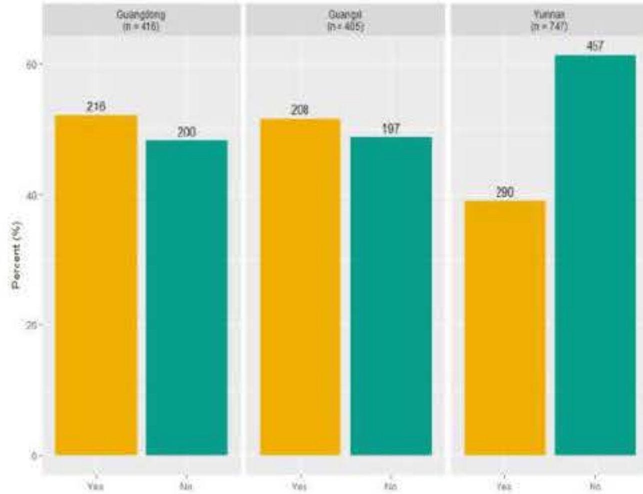


Figure 7: *Location where care was usually received for illness or infection.*

All survey respondents were asked whether they had experienced an unusual illness in their lifetime and in the past year, defined by a series of the most common symptoms associated with encephalitis, hemorrhagic fever (HF), severe acute respiratory infection (SARI), and influenza-like illness (ILI). Additional symptoms that were asked about included: fever with diarrhea or vomiting; fever with rash; and, persistent rash or sores on skin. Respondents were not restricted to selecting one illness and could provide multiple responses.

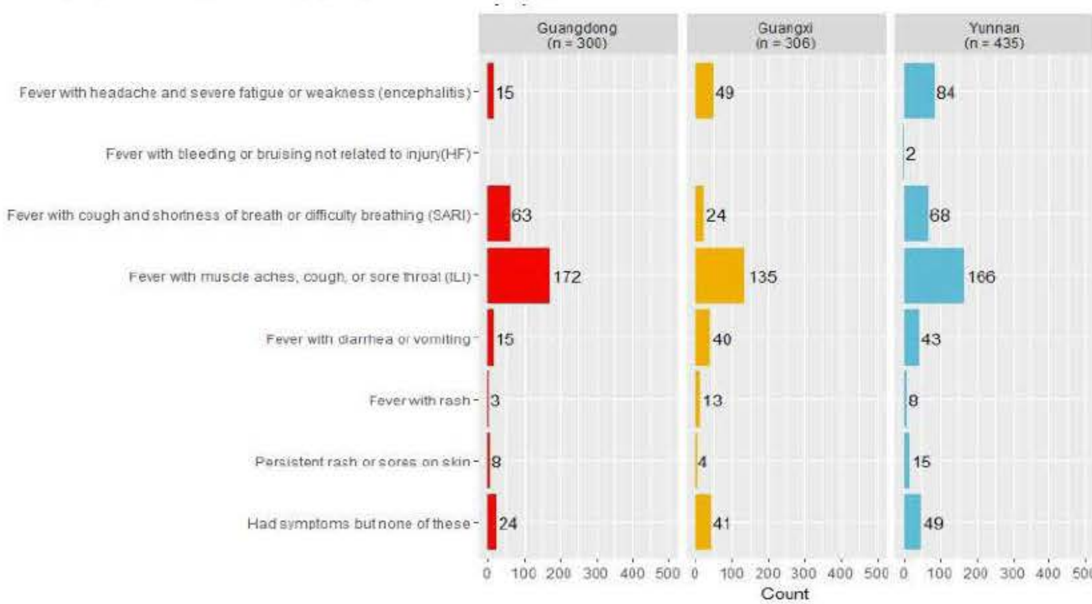
The proportion of respondents who had an unusual illness with any of the above-mentioned symptoms in their lifetime varied slightly by province. Between the three provinces, Yunnan had the fewest number of respondents who reported experiencing the symptoms provided (38.8%), compared to Guangdong and Guangxi (51.9% and 51.3%, respectively). Yunnan was also the only province where less than half of the respondents reported experiencing the symptoms provided **(Fig. 8)**.

Figure 8: *Respondent's experience of unusual illnesses.*



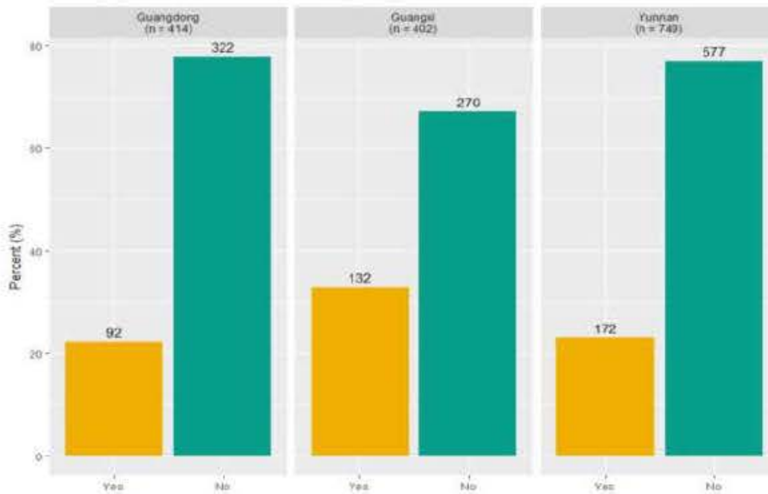
Across all three provinces, among those who had experienced any symptoms of unusual illness <u>in their lifetimes</u>, those associated with ILI were the most commonly reported. In Guangdong province, this was followed by symptoms associated with SARI, then by other symptoms not mentioned in the survey. In Guangxi province, the second most reported symptoms were ones associated with encephalitis, followed by other symptoms not mentioned in the survey. Similarly, in Yunnan, symptoms associated with encephalitis were the second most commonly reported, but this was followed by symptoms associated with SARI **(Fig. 9)**.

Figure 9: *Symptoms reported by people who had experienced unusual illness in their lifetime.*



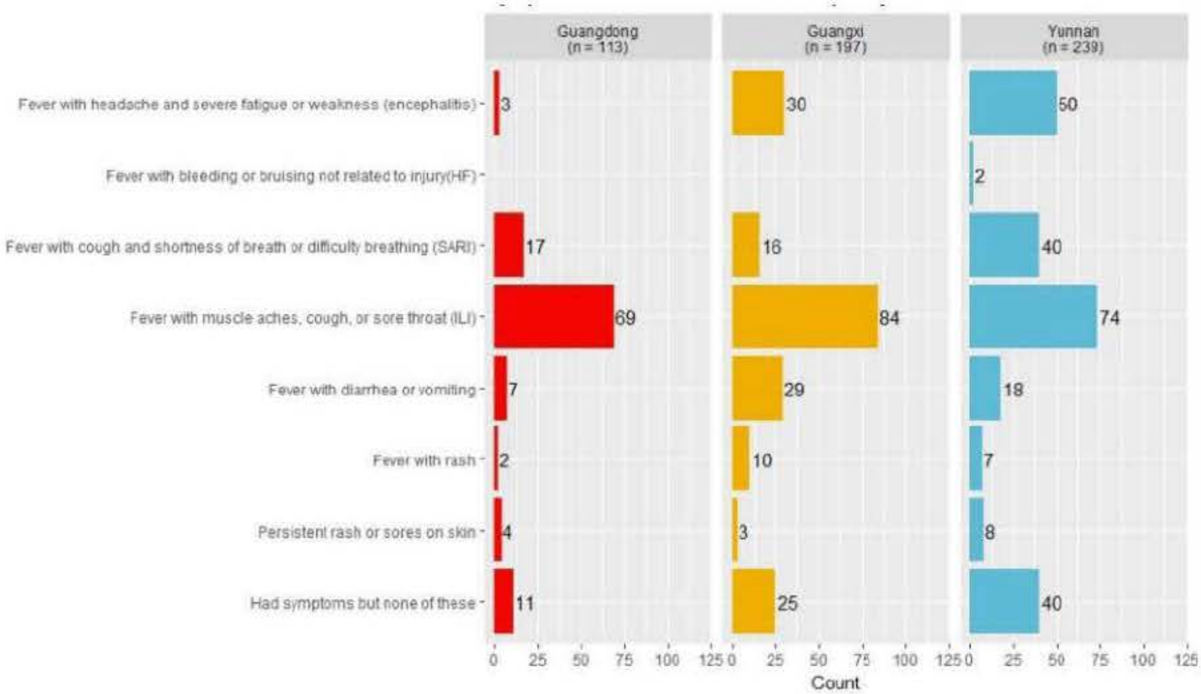
In each province, just under one-third of respondents who experienced the symptoms associated with an unusual illness in their lifetime indicated experiencing any of the symptoms in the past year – 22.2% in Guangdong, 32.8% in Guangxi and 23.0% in Yunnan **(Fig. 10)**.

Figure 10: *Whether respondents had experienced symptoms associated with an unusual illness, in the past year.*



Of the respondents who reported having symptoms of unusual illness <u>in the past year</u>, across all three provinces, symptoms associated ILI were the most commonly reported. In Guangdong province, this was followed by symptoms associated with SARI then by other symptoms not provided in the survey. In Guangxi, symptoms associated with ILI were followed by symptoms associated with encephalitis, then by fever with diarrhea or vomiting. In Yunnan, symptoms associated with ILI were followed by symptoms associated with encephalitis, then by both SARI and other symptoms not provided in survey **(Fig. 11)**.

Figure 11: *Symptoms experienced by those reporting unusual illness in the past year.*



When respondents were asked what caused the symptoms associated with unusual illness experienced in the past year, 64.4% in Guangxi (85 of 132 respondents), and 50.0% in both Guangdong and Yunnan (46 of 92 respondents and 86 of 172, respectively), said they did not know the cause **(Fig. 12)**. Only one respondent in Guangxi said their symptoms were due to

contact with animals (wild animals, specifically). Two respondents in Guangdong and one respondent in Guangxi said their symptoms were due to contact with animals (non-wild animals, specifically), whereas none of the respondents in Yunnan attributed their cause to contact with animals.

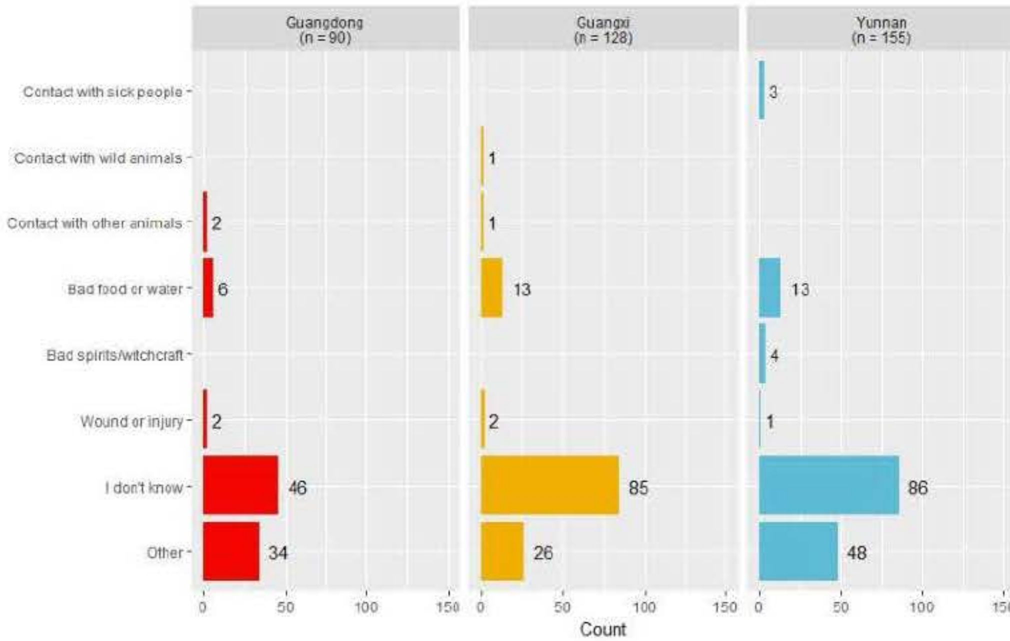
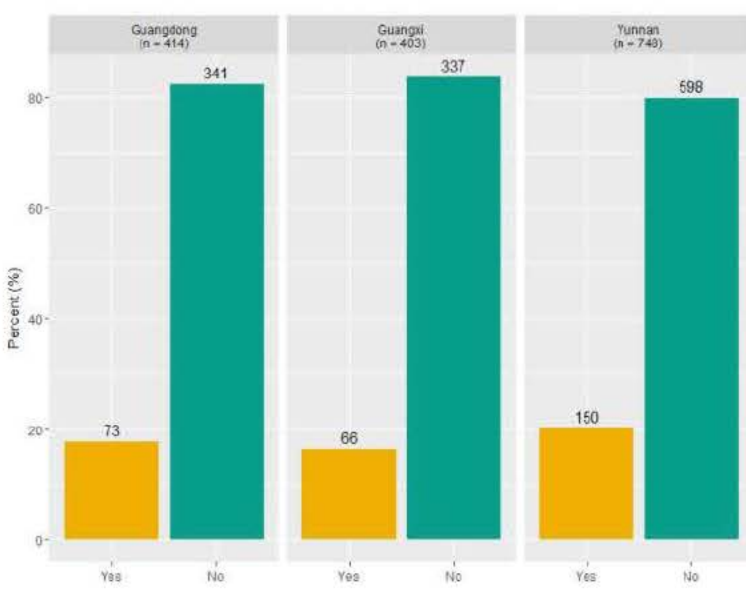


Figure 12: *Reported cause of sickness in the past year.*

Respondents reporting an unusual illness in the past year were asked if any of the people they lived with in the past year had symptoms similar to theirs, to assess possibilities of transmission among household members. Most respondents did not, across all three provinces: 82.4% in Guangdong, 83.6% in Guangxi and 79.9% in Yunnan **(Fig. 13)**.

Figure 13: *Whether household members had similar symptoms of unusual illness, in the past year*



Of the household members who experienced symptoms of unusual illness in the past year, the most commonly reported symptoms were those associated with ILI **(Fig. 14)**.

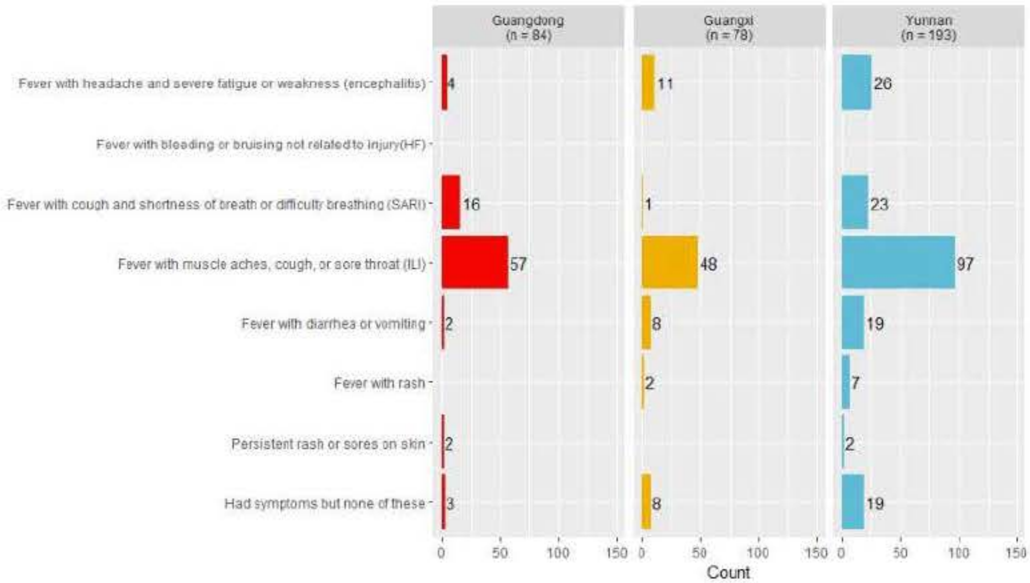


Figure 14: *Symptoms of household members who were ill, in past year.*

Respondents were also asked if any members of their household who experienced symptoms of unusual illness died as a result of their illness in the past year. Across all the three provinces, almost none had died from these illnesses **(Fig. 15)**.

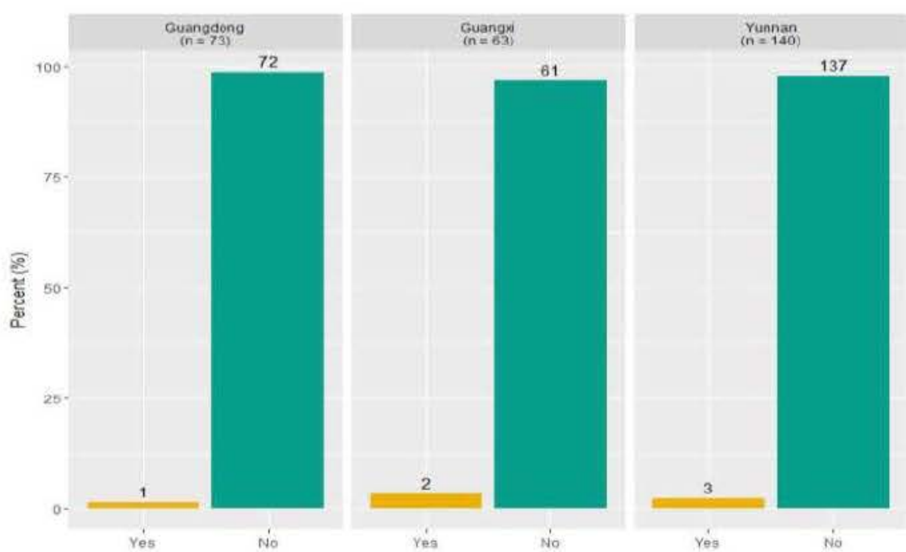


Figure 15: *Whether household members died from illness, in the past year.*

Contact with Animals

All respondents were asked about various types of animal contacts in their lifetime and in the past year. More than two-thirds of the respondents across all provinces, as well as in each of the provinces, reported raising an animal within their lifetime (71.2% in Guangdong, 77.7% in Guangxi, and 97.7% in Yunnan). More than half of the respondents in each province reported having animals come inside their dwellings (83.1 % in Guangdong, 60.2% in Guangxi, and 92.5% in Yunnan). More than half of respondents in each province reported handling live animals (51.5 % in Guangdong, 56.9% in Guangxi, and 62.9% in Yunnan) **(Table 1)**. Respondents from Yunnan had more types of contact with animals in their lifetime than those from Guangdong and Guangxi. With the exception of cooking or handling meat, organs, or blood from a recently killed animal and being scratched or bitten by an animal, the proportion of respondents from Yunnan who engaged in all types of animal activities was higher than the other provinces.

Type of animal contact (past year)	Guangdong		Guangxi		Yunnan	
	(n)	(%)	(n)	(%)	(n)	(%)
Lived with an animal as a pet	43	100 %	72	98.6 %	335	100 %
Handled live animals	212	100 %	226	98.3 %	332	99.7 %
Raised a live animal	296	100 %	312	99.4 %	518	99.8 %
Shared water source with animals for washing	47	100 %	19	95.0 %	97	100 %
Seen animal feces in or near food before you have eaten it	18	100 %	15	93.8 %	43	100 %
Eaten food after an animal has touched or damaged it	6	100 %	6	100 %	29	100 %
Animals come inside the dwelling where you live	345	100 %	239	98.0 %	493	100 %
Cooked or handled meat, organs, or blood from a recently killed animal	333	100 %	144	97.3 %	412	100 %
Eaten raw or undercooked meat or organs or blood	2	100 %	25	89.3 %	65	98.5 %
Eaten an animal that was not well/sick	--	--	1	100 %	6	100 %
Found a dead animal and collected it to eat, share, or sell	--	--	3	100 %	10	100 %
Been scratched or bitten by an animal	1	100 %	31	100 %	28	96.6 %
Slaughtered an animal	145	100 %	69	98.6 %	303	100 %
Hunted or trapped an animal	9	100 %	4	100 %	22	95.7%

Table 1: *Types of animal contact, within a respondent's lifetime.*

Respondents who reported having animal contact in their lifetime were also asked to indicate if they had the same type of animal contact in the past year **(Table 2)**. In the past year, across all three provinces and in each province, almost all respondents engaged in all contact types with the exception of eating an animal that was not well/sick, and finding a dead animal and collecting it to eat, share, or sell (0% for both in Guangdong).

Type of animal contact (lifetime)	Guangdong		Guangxi		Yunnan	
	(n)	(%)	(n)	(%)	(n)	(%)
Lived with an animal as a pet	43	10.4 %	73	18.1 %	335	62.9 %
Handled live animals	212	51.5 %	230	56.9 %	334	62.8 %
Raised a live animal	296	71.2 %	314	77.7 %	521	97.7 %
Shared water source with animals for washing	47	11.5 %	21	5.2 %	97	18.2 %
Seen animal feces in or near food before you have eaten it	18	4.4 %	16	3.9 %	43	8.1 %
Eaten food after an animal has touched or damaged it	6	1.5 %	6	1.5 %	29.0	5.4 %
Animals come inside the dwelling where you live	345	83.1 %	244	60.2 %	493	92.5 %
Cooked or handled meat, organs, or blood from a recently killed animal	333	80.4 %	148	36.7 %	413	77.5 %
Eaten raw or undercooked meat or organs or blood	2	0.5 %	28	6.9 %	68	12.8 %
Eaten an animal that was not well/sick	--	--	1	0.3 %	6	1.1 %
Found a dead animal and collected it to eat, share, or sell	--	--	3	0.7 %	10	1.9 %

Table 2: *Types of animal contact, in past year.*

Respondents who had animal contact in the past year were asked to identify the animals involved in the interaction. *(Figs. 16-26, below: the first two figures are enlarged to show row labels, which are identical for all).* Cats and dogs were the most common pets reported across all provinces and in each province **(Fig. 16b)**.

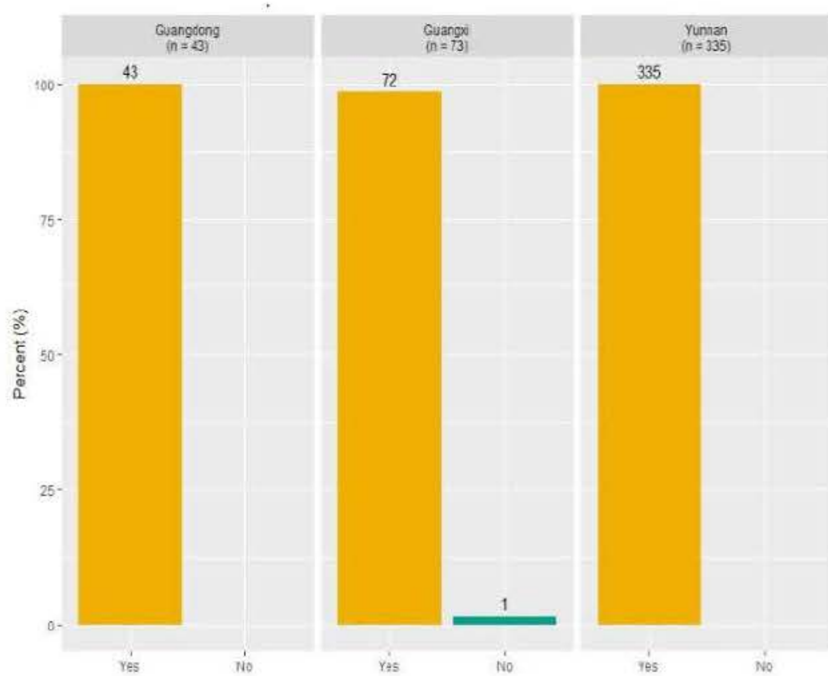
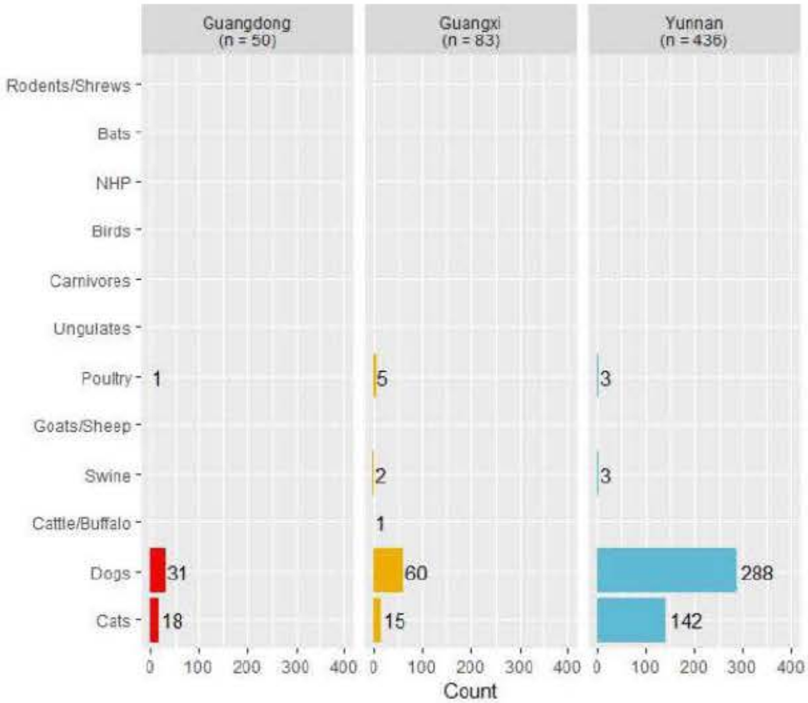


Figure 16a (top) & b (below): *(a) Whether respondents had lived with an animal as a pet, in the past year, and (b) among those who had, types of animal kept as pets.*



Poultry was the most common type of animal handled across all provinces as well as in each province, with 96.2%, 90.3%, and 92.8% of respondents handling animals in Guangdong, Guangxi and Yunnan, respectively **(Fig. 17b)**.

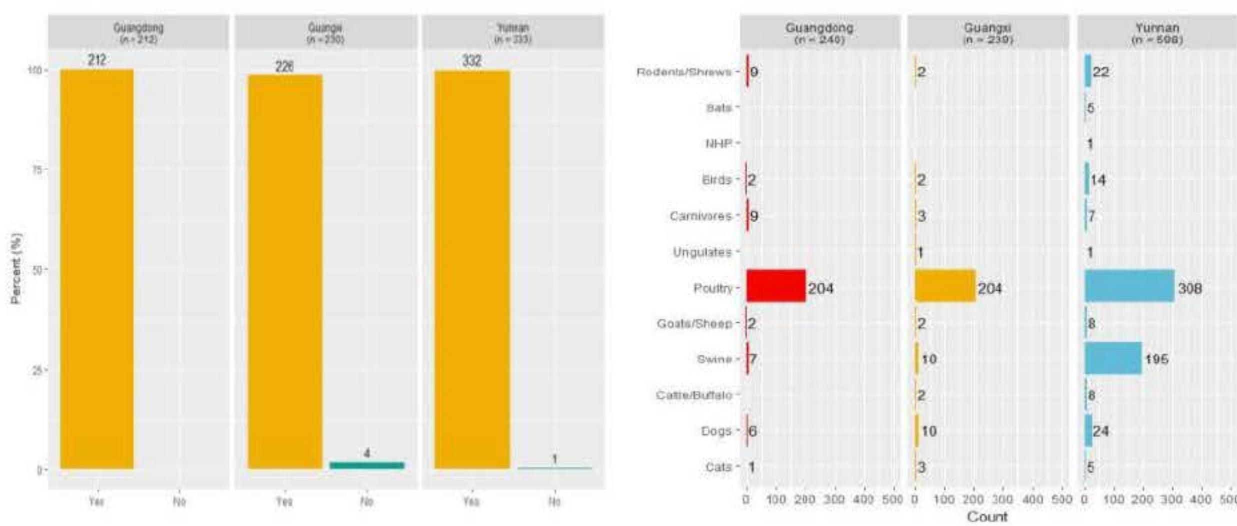
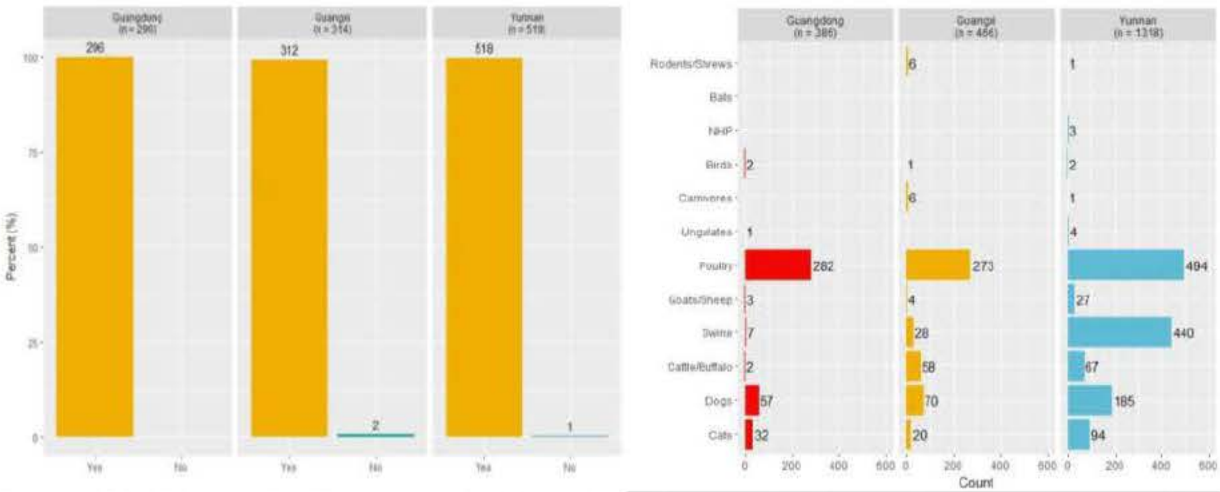


Figure 17a & b: *(a) Whether respondents had handled live animals, in the past year, and (b) among those who had, types of live animals handled.*

Poultry was also the most commonly raised animal in each of the three provinces; 95.3%, 87.5%, 95.4% in Guangdong, Guangxi, and Yunnan, respectively **(Fig. 18b)**.



Figures 18a & b: *(a) Whether respondents had raised live animals in the past year, and (b) among those who had, types of animals raised.*

In all three of the provinces, the most common type of animals found in respondent dwellings were rodents or shrews. In Guangdong and Yunnan, birds were the second most common animal type found in dwellings. In Guangxi province, birds along with poultry were the second most common animal type. Respondents in Guangdong and Yunnan reported that all 12 animal taxa had come inside their dwellings in the past year. Taxa seen in the dwellings of respondents from Guangdong and Yunnan and not Guangxi were non-human primates, ungulates, goats or sheep, swine, and cattle or buffalo **(Fig. 20b)**.

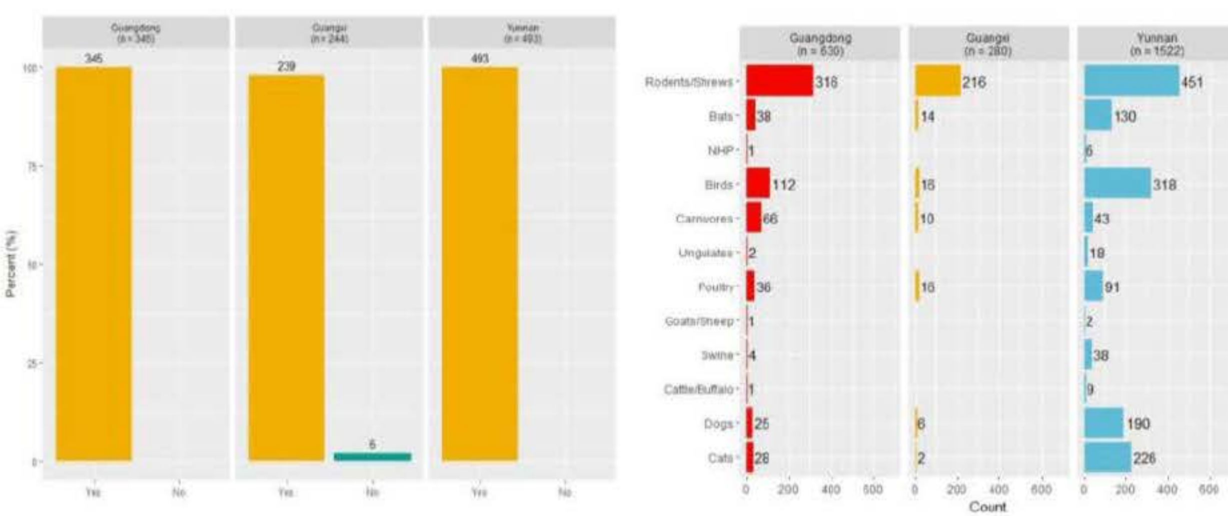


Figure 19a & b: *(a) Whether respondents had animals come inside dwelling, in the past year, and (b) among those who had, types of animals in dwelling.*

Almost all of the respondents who said they have cooked or handled meat, organs, or blood in their lifetime reported doing so in the past year. Common animal types that were cooked handled included poultry and swine in all three provinces **(Fig. 20)**.

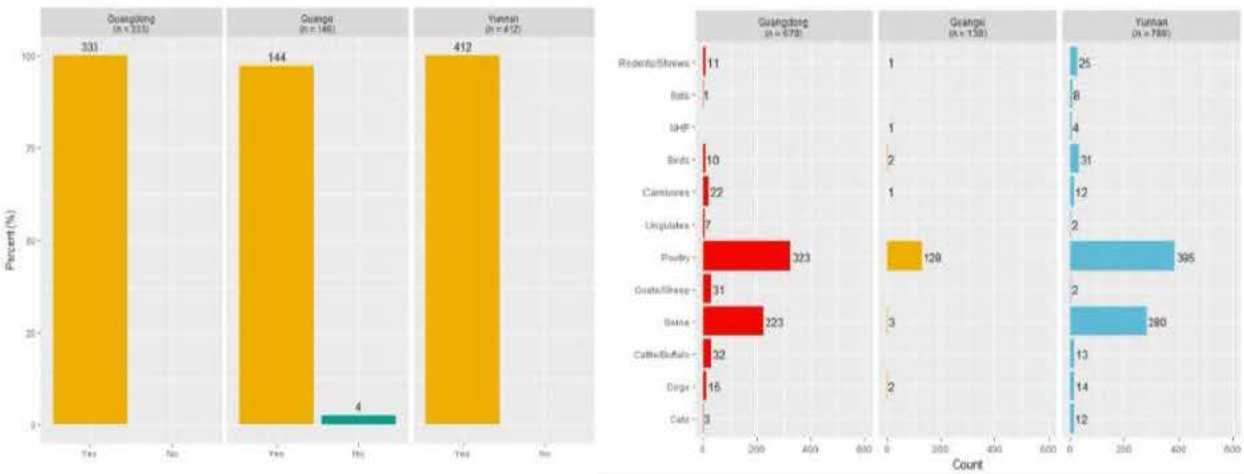


Figure 20a & b: *(a) Whether respondents had cooked or handled meat, organs or blood from a recently killed animal, in the past year, and (b) among those who had, types of animals whose meat, organs or blood was cooked or handled.*

More respondents in Yunnan reported eating raw or undercooked meat compared to respondents in Guangdong and Guangxi **(Fig. 21)**. In Yunnan, 96% of respondents who ate raw or undercooked meat in their lifetime did so in the past year. The types of animal products that were eaten raw or undercooked by respondents in Yunnan were mostly from swine. In Guangxi, the most commonly reported type of animal meat that had been eaten raw or undercooked was that of carnivores.

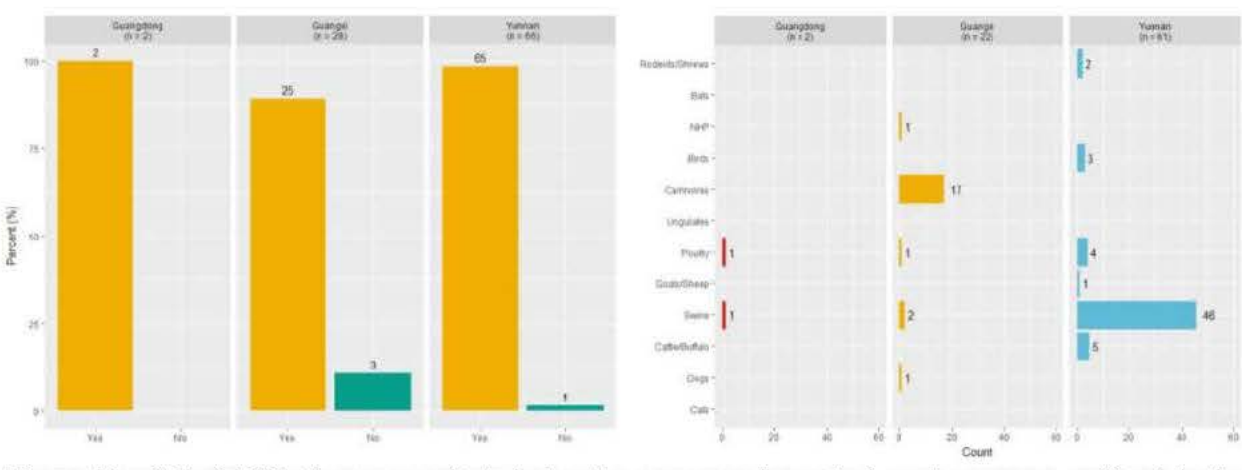


Figure 21 a & b: *(a) Whether respondents had eaten raw or undercooked meat or organs or blood, in the past year, and (b) among those who had, types of animals whose meat, organs or blood were eaten raw or undercooked.*

Across all provinces, a total of 13 respondents in Guangxi and Yunnan indicated that they collected an animal that was found dead to eat, share or sell. In Guandong, no respondents reported finding a dead animal and collecting it to eat, share, or sell. The most common type of animal collected across all provinces in aggregate was poultry. In Yunnan, poultry was the most common type of animal found dead and collected to eat, share or sell (80.0%), whereas dogs were the most common type in Guangxi (66.7%) **(Fig. 22)**.

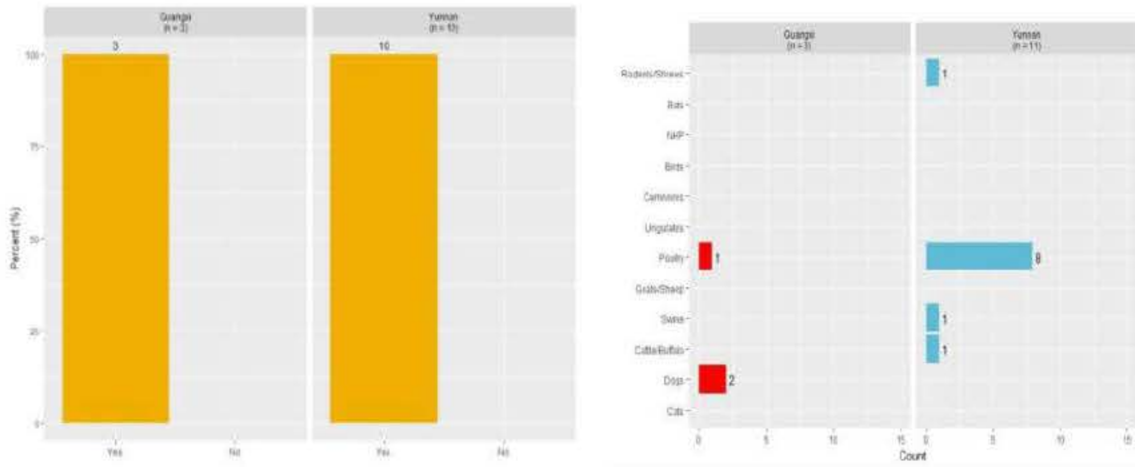


Figure 22 a & b: *(a) Whether respondents had found a dead animal and collected it to eat, share, or sell, in the past year, and (b) among those who had, types of animals that were found dead and collected to eat, share, or sell.*

In each province, almost all of the respondents who indicated being scratched or bitten by an animal in their lifetime said it occurred in the past year (100% in Guangdong, 98.6% in Guangxi, and 100% in Yunnan). In both Guangxi and Yunnan, dogs were the common type of animal that respondents said they were scratched or bitten by (64.5% in Guangxi and 50.0% in Yunnan). Cats were the second most common in Guangxi and Yunnan (9.6% in Guangxi, and 28.5% in Yunnan). Across all three provinces, only one respondent from Yunnan said that they were scratched or bitten by a bat **(Fig. 23)**.

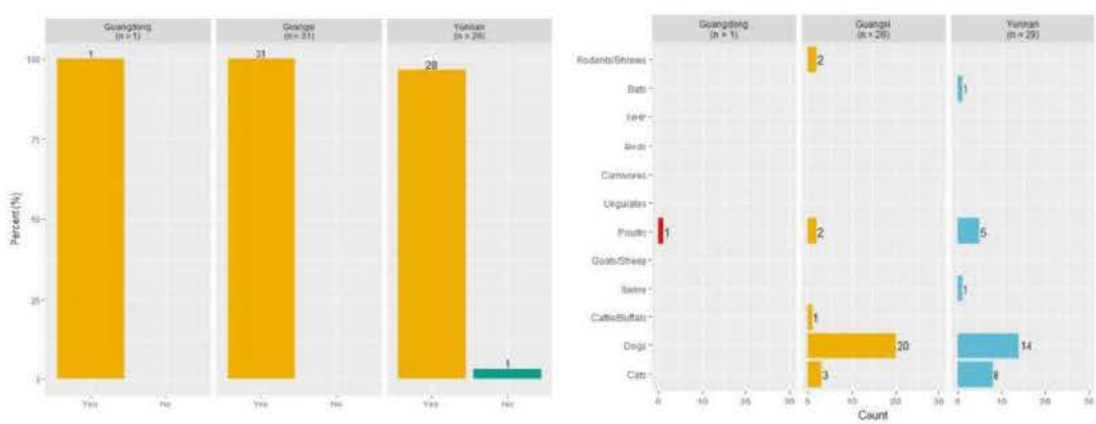


Figure 23 a & b: *(a) Whether respondents had been scratched or bitten by an animal, in the past year, and (b) among those who had, types of animals that scratched or bit respondents.*

Poultry was the most common type of animal slaughtered during the past year across all provinces as well as in each province (95.8% in Guangdong, 79.7% in Guangxi, and 94.1% in Yunnan). In addition to poultry, respondents in Yunnan also commonly only slaughtered swine (43.9%), compared to 1.4% in Guangdong and 7.3% in Guangxi **(Fig. 24)**.

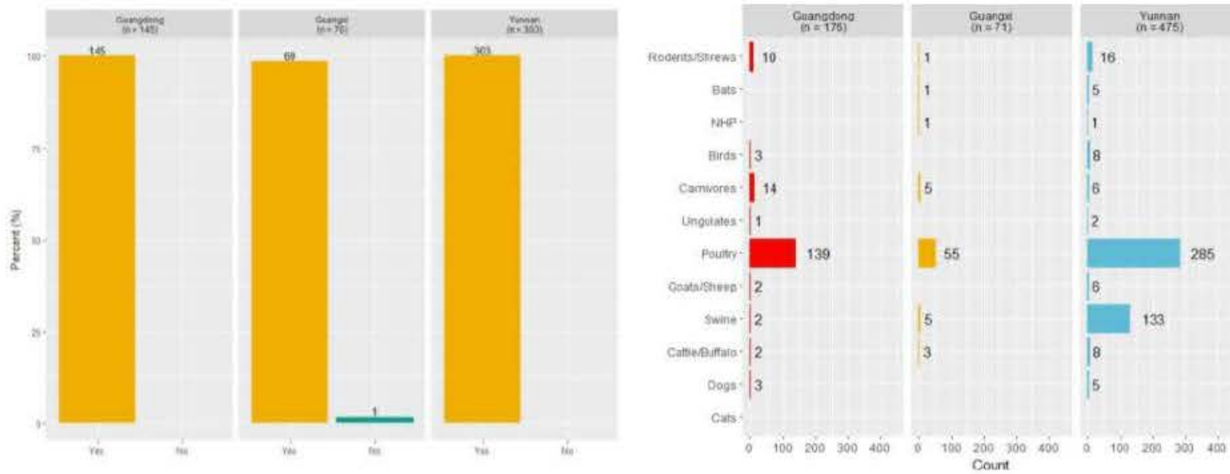


Figure 24 a & b: *(a) Whether respondents had slaughtered an animal, in the past year, and (b) among those who had, types of animals slaughtered.*

Carnivores were the most common taxa of animals hunted or trapped in the past year, in Guangdong and Guangxi. In Yunnan, rodents or shrews and birds were reported as the most common. Bats, non-human primates and dogs were animal types hunted by respondents in Yunnan but not by respondents in Guangdong and Guangxi **(Fig. 25)**.

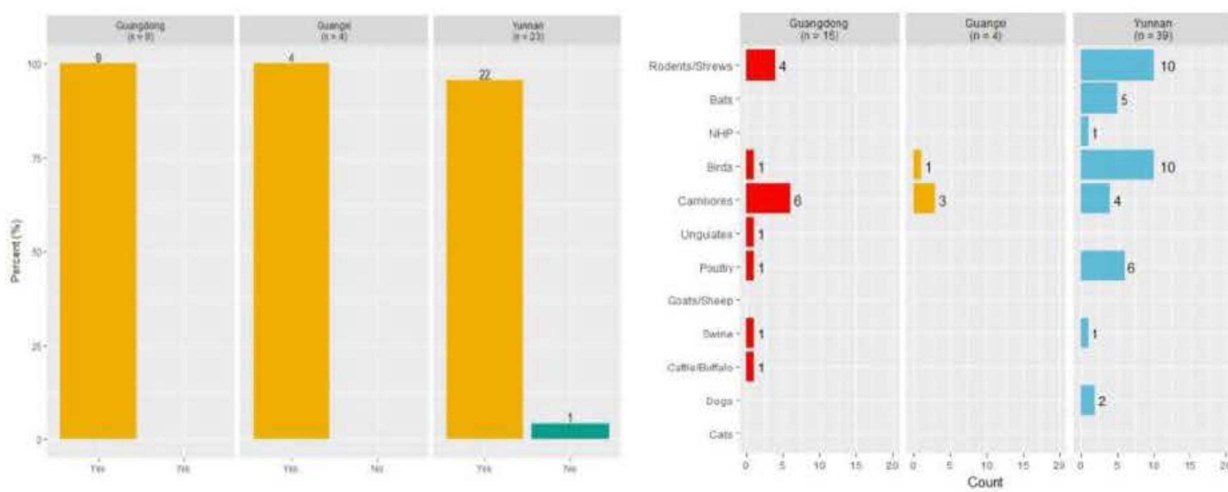


Figure 25 a & b: *(a) Whether respondents had hunted or trapped an animal, in the past year, and (b) among those who had, types of animals hunted or trapped.*

In examining bat-specific contact, across all provinces and within each province, the most common interaction with bats was finding them inside their houses. Respondents in Yunnan also hunted/trapped and handled bats, and were scratched/bitten by bats, whereas these did not occur in Guangdong or Guangxi **(Fig. 26)**.

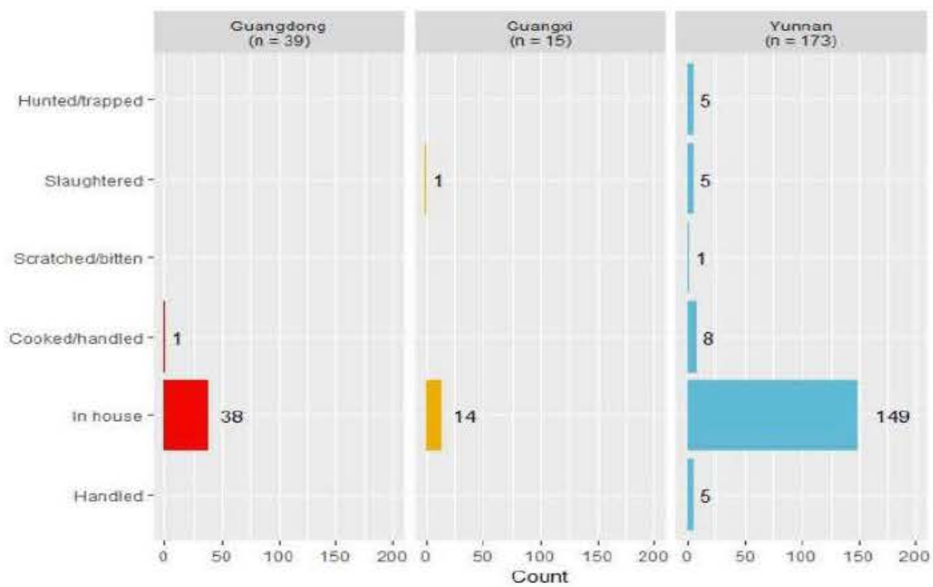


Figure 26: *Types of bat contact.*

After respondents were asked about their contact with wildlife and livestock, they were asked about their knowledge of whether animals can spread diseases and whether they were worried about diseases and disease outbreaks at wet markets. The proportion of respondents who thought that animals can spread disease was highest in Guangdong province (72.3%). In Guangxi and Yunnan, the proportion of those who thought animals could spread disease compared to those who thought that they did not were roughly equivalent – 47.5% versus 50.7% in Guangxi and 49.2% versus 49.3% in Yunnan **(Fig. 27)**.

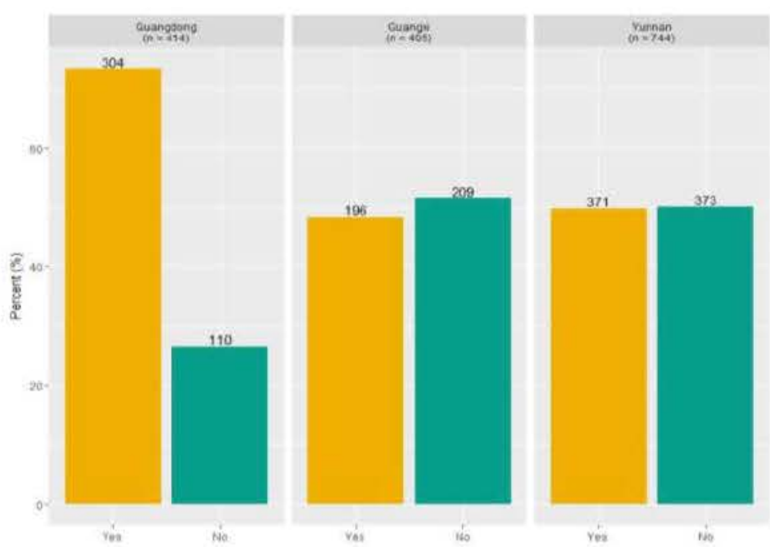


Figure 27: *Whether respondents thought that animals can spread disease.*

Similarly, when respondents were asked about whether they were worried about diseases or disease outbreaks in animals at wet markets, Guangdong had the highest proportion of respondents who said they were worried (67.3%). In both Guangxi and Yunnan, the proportion of respondents that was not worried (57.5% and 51.5%, respectively) was higher than the proportion that was worried **(Fig. 28)**

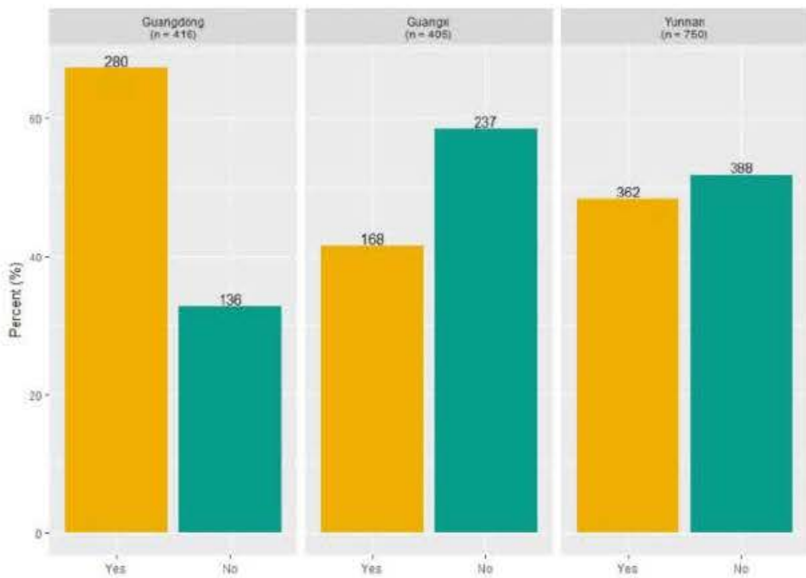


Figure 28: *Whether respondents were worried about diseases or disease outbreaks in animals at wet markets.*

Serological Evidence of Bat SARS-related CoV Infection in Humans

Respondents were asked to provide a biological sample to assess whether SARS-CoV spillover had occurred at the high-risk location where the survey has been implemented. A total of 1,530 serum samples were collected from 2016 to 2017 from individual residents in villages close to bat caves where coronaviruses were previously detected.

We developed an ELISA serology test using the purified NP protein of MERS-CoV, SARSr-CoV, HKU9 CoV and HKU10 CoV as coating antigen respectively and using Anti-Human IgG Monoclonal antibody as secondary antibody. All sera were screened for antibodies against these 4 bat-origin coronaviruses. Anti-SARSr-CoV NP IgG was detected in 10 samples, and 6 samples were positive for IgG against HKU10 NP. The 16 ELISA positive samples were further tested by confirmatory western blot, 7 samples from Yunnan province were confirmed positive for anti-SARSr-CoV, two samples (one from Guangdong province and one Guangxi province) were confirmed positive for anti-HKU10 **(Table 3)**.

Locations		Sample No.	NP Antibody Positive No.			
			HKU9 CoV	MERS CoV	SARSr-CoV	HKU10 CoV
Yunnan (2016)	Jinning	209			*6	
	Mengla	168			2 (*1)	
	Jinghong	212				2
	Lufeng	144				
Guangdong (2016)	Zengcheng	234			1	2
	Ruyuan	179				
Guangxi (2017)	Mashan	160			1	
	Guilin	224				*2
Total		1,530	0	0	*7	*2

Table 3 *Results of ELISA testing of human sera for antibodies to 4 different bat CoV species (*confirmed with western blot).*

<u>*Links Between ELISA Results and Behavior*</u>

Only one out of the seven SARS-related CoV seropositive respondents said that they had an unusual illness in their lifetime with reported symptoms similar to encephalitis or neural involvement. Two of the respondents said they had experienced symptoms in the past year with only one respondent specifying that they experienced epigastric pain and dizziness. The seven seropositive SARSr-CoV respondents reported various types of animal contacts in the past year. Three had lived with an animal as a pet, four handled a live animal, four raised a live animal, five saw animals inside their dwellings, five had cooked or handled meat, organs, or blood from a recently killed animals, one ate an animal that they knew was not well or sick, one was scratched or bitten by an animal, and four had slaughtered an animal. The only bat contact reported was by one respondent who saw a bat in their dwelling.

Both of the respondents who tested positive for HKU10-CoV antibodies said they had experienced an unusual illness in their lifetime, with symptoms associated with encephalitis and SARI. Neither respondent had experienced any symptoms of unusual illness in the past year. Both had reported handling and raising animals, with one indicating they saw animals come inside their dwelling, and one indicating cooking or handling meat, organs, or blood from a recently killed animal. No bat contact was reported by either of the respondents. Overall, five of the total nine SARS-related CoV and HKU10-CoV seropositive respondents reported being worried about disease or disease outbreaks at wet markets. Seven of the nine reported purchasing live animals from a wet market.

Specific Aim 1: Summary of Key Findings

Our analysis of the key risk factors relating to potential viral zoonotic disease spillover in China indicated some notable differences among the respondents in Guangdong, Guangxi, and Yunnan. With respect to demographic factors, Guangxi fared the lowest on key socio-economic

status indicators when compared to Guangdong and Yunnan provinces as reflected by the higher proportion of respondents in Guangxi living under the poverty level.

When assessing the type of animal contact and the associated animal taxa over the course of a respondent's lifetime, the results show that respondents in Yunnan engaged in greater contact with animals then those from Guangdong and Guangxi. For example, for 12 of the 14 animal contact types, a higher proportion of Yunnan respondents engaged in these respective activities than in Guangdong and Guangxi. Respondents in Yunnan also reported hunting bats, dogs, and non-human primates which were not reported to being hunted in Guangdong and Guangxi. Swine contact was higher in Yunnan for handling, raising, and slaughtering activities. When examining the various types of animal contact associated with bats only, our results also show that Yunnan respondents reported more varied types of contact with bats. Respondents in Yunnan indicated handling, being scratched by, slaughtering, and hunting bats, but these interactions did not occur in Guangdong or Guangxi. Additional analyses that examine predictors of animal contact in each province will be the focus of human behavioral analyses in Year 5 of the study.

Even though our sample population lives in areas that have dense and diverse bat populations, our results show an overall low proportion of respondents reporting hunting and trapping bats in all three provinces. The low proportion of hunting practice could be attributed to the success of conservation enforcement efforts undertaken by the government. These efforts may have effectively reduced the illegal practice of hunting wildlife or, as a consequence, moved the activity underground which made respondents less forthcoming about revealing their engagement in such practices. Further investigation into the potential causes is also warranted.

Our analyses also reveal differences in perceptions associated with zoonotic disease spillover between Guangdong, and Guangxi and Yunnan. For example, the proportion of respondents who thought that animals can spread disease was highest in Guangdong province at 72.3%, as compared to Guangxi (48.3%) and Yunnan (49.9%). Moreover, about two-thirds of respondents in Guangdong were worried about diseases and disease outbreaks in wet markets. These differences in perception observed in Guangdong compared to Guangxi and Yunnan could potentially be attributable to a heightened awareness of zoonotic disease emergence due to the 2001 SARS outbreak.

Finally, our serological testing results provide the first evidence ever of a bat SARSr-CoV spilling over into people in the wild. All of the SARSr-CoV positive individuals were from Yunnan province, which is the site of a cave in which we have identified a large diversity of SARSr-CoVs within the virome of which every genetic element of SARS-CoV can be identified. These findings warrant further investigations into the type of exposures that may have contributed to bat SARS-related CoVs to infect humans in this particular region. **They also highlight this region as a hotspot for SARSr-CoV future spillover risk.**

Specific Aim 2: Receptor evolution, host range and predictive modeling of bat-CoV emergence risk

Bat CoV PCR Detection and Sequencing from Live-Sampled Bat Populations

We collected rectal swab and oral swab samples from 671 individual bats from 20 species in Guangdong and Guangxi provinces in southern China in Year 4 **(Table 4)**. 671 rectal swab samples were tested for CoV RNA and 154 (23.0%) were positive **(Table 5)**.

Date of Sampling	Sampling Locations	Rectal swabs	Oral swabs
May 10th 2017	Hezhou, Guangxi	6	6
May 11-12th 2017	Chongzuo, Guangxi	67	67
May 13th 2017	Nanning, Guangxi	66	66
May 17th, 2017	Beihai, Guangxi	23	23
May 19th 2017	Chongzuo, Guangxi	36	36
May 21st 2017	Yangshan, Qingyuan, Guangdong	46	46
May 22nd, June 7th 2017	Huidong, Huizhou, Guangdong	103	103
June 9th 2017	Nanning, Guangxi	71	71
June 9th 2017	Ningming, Chongzuo, Guangxi	63	63
September 10th 2017	Huidong, Huizhou, Guangdong	100	100
September 11th 2017	Yingde, Guangdong	90	90
Total		**671**	**671**

Table 4. *Bat samples collected for CoV surveillance in Year 4*

Species	Guangdong	Guangxi	Total
Rhinolophus sinicus	**9**/27	6	**9**/33
Rhinolphus rex		4	4
Rhinolophus pusilus	1	2	3
Rhinolophus pearsoni	5		5
Hipposideros armiger	24	8	32
Hipposideros larvatus	9	9	18
Hipposideros pomona		20	20
Hipposideros pratti	26		26
Aselliscus stoliczkanus		1	1
Miniopterus fuliginosus	1		1
Miniopterus pusillus	**29**/39		**29**/39
Myotis chinensis	**2**/27		**2**/27
Myotis daubentonii	2		2
Myotis ricketti	**86**/178		**86**/178
Pipistrellus abramus		2	2
Pipistrellus pipistrellus		2	2
Scotophilus kuhli		**24**/137	24/137
Tylonycteris pachypus		**4**/115	**4**/115
Tylonycteris robustula		3	3
Cynopterus sphinx		23	23
Total	**126/339**	**28/332**	**154/671**

Table 5*. Number of bat specimens tested and positive (bold) in Year 4*

A high prevalence of HKU6-related coronaviruses (48.3%), Scotophilus coronavirus 512 (17.5%), and coronavirus 1B (71.8%) was detected in *Myotis ricketii*, *Schotophilus khulii* and *Miniopterus pusillus*, respectively. SARS-related coronaviruses and HKU2-related coronaviruses were discovered in 4 and 5 *Rhinolophus sinicus* samples respectively from Guangdong. HKU4 coronaviruses were identified in 4 *Tylonycteris pachypus* from Guangxi **(Fig. 29)**.

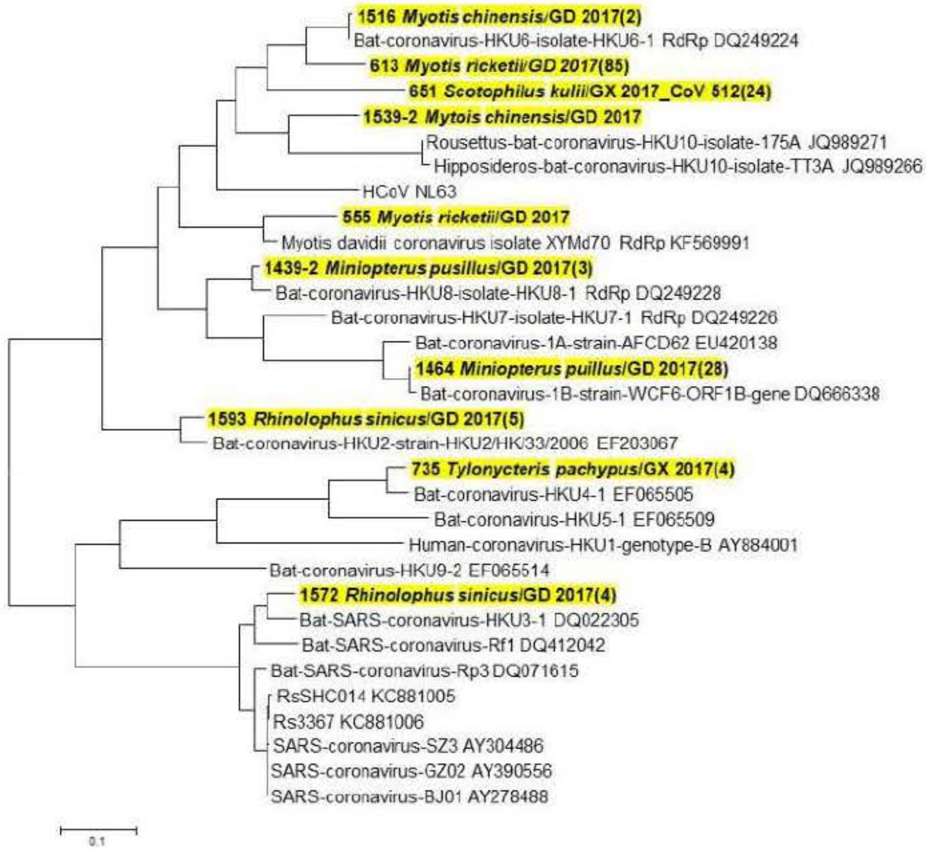


Figure 29: *Phylogenetic analysis of partial RdRp gene of CoV (440-nt partial sequence)*

Genetic Diversity and Genomic Characterization of Betacoronaviruses in Fruit Bats
In Year 4, we analyzed the genetic diversity of betacoronaviruses we have detected since 2009 in different species of fruit bats in Yunnan province, including *Eonycteris spelaea*, *Rousettus leschenaultia* and an unclassified *Rousettus* species. These viruses are classified into two betacoronavirus species, HKU9-CoV and GCCDC1-CoV. All HKU9-related viruses (n=46) were found in *Rousettus* spp. bats while GCCDC1-related viruses (n=13) from *E. spelaea*. Phylogenetic analysis of the full-length N gene suggests that HKU9-related CoVs are highly diverse and divided into 5 lineages with previously reported strains, and the GCCDC1-related CoVs were more similar between each other **(Fig. 30)**.

The full-length genome sequence of a novel HKU9-related CoV termed 2202 was determined. It shares 83% nt identity with other HKU9 strains, with the most divergent regions located in the S

protein, but shares only 68% aa identity with those of other HKU9 strains. Virus quantification revealed that intestine was the primary infected organ for HKU9-related CoVs while kidney and lungs could also be target tissues, suggesting potential for spillover through oral-fecal, respiratory, or uro-genitary routes.

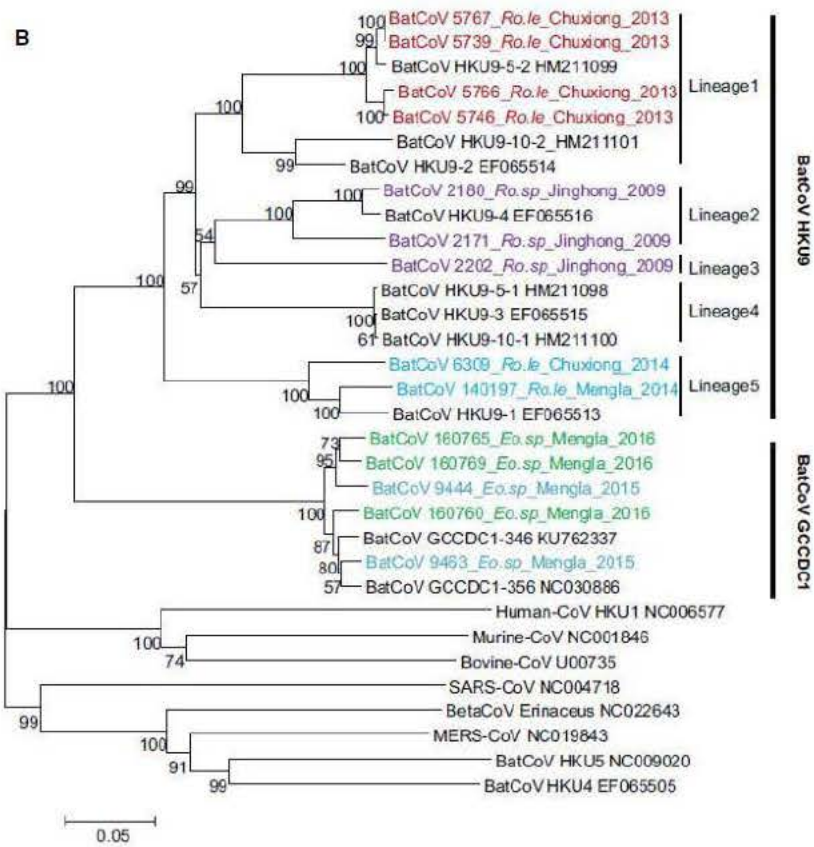


Figure 30. *Phylogenetic analysis of full-length N gene of HKU9 and GCCDC1 CoVs*

Bat Coronavirus Host-Virus Phylogeography in China

We used discrete ancestral character state reconstruction to estimate viral history and reconstructed the inferred bat host genus for each node within the phylogenetic tree **(Figs. 31, 32)**. The color of tree branches indicates the inferred ancestral host bat genus for the reconstructed phylogeny. *Rhinolophus* is the inferred ancestral host of lineages B and C (SARS-like CoVs and MERS-like CoVs, respectively). This genus played an important role in the diversification of Beta-CoVs. A larger host diversity is observed for Alpha-CoVs. Our dataset for this analysis includes all CoV RdRp sequences isolated from bat specimens collected by our team from 2008-2015 (Alpha-CoVs: n = 491 – Beta-CoVs: n = 326), including those collected under prior NIAID funding (1 R01 AI079231), funding from Chinese Federal Agencies, and a large majority from our current NIAID project. All Chinese bat CoV RdRp sequences available in GenBank were also added to our dataset (Alpha-CoVs: n = 226 – Beta-CoVs: n = 206).

Phylogenetic trees were reconstructed for Alpha- and Beta-CoVs separately using Bayesian inference (BEAST 1.8).

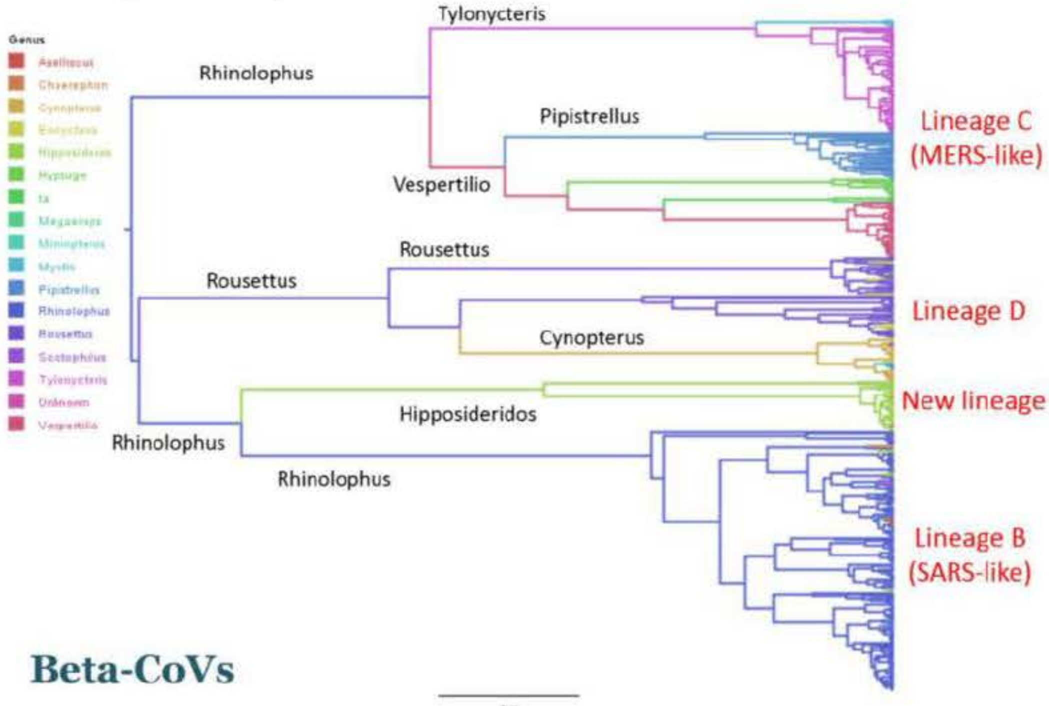


Figure 31. *Ancestral host reconstruction for Beta-CoVs, at a host genus level.*

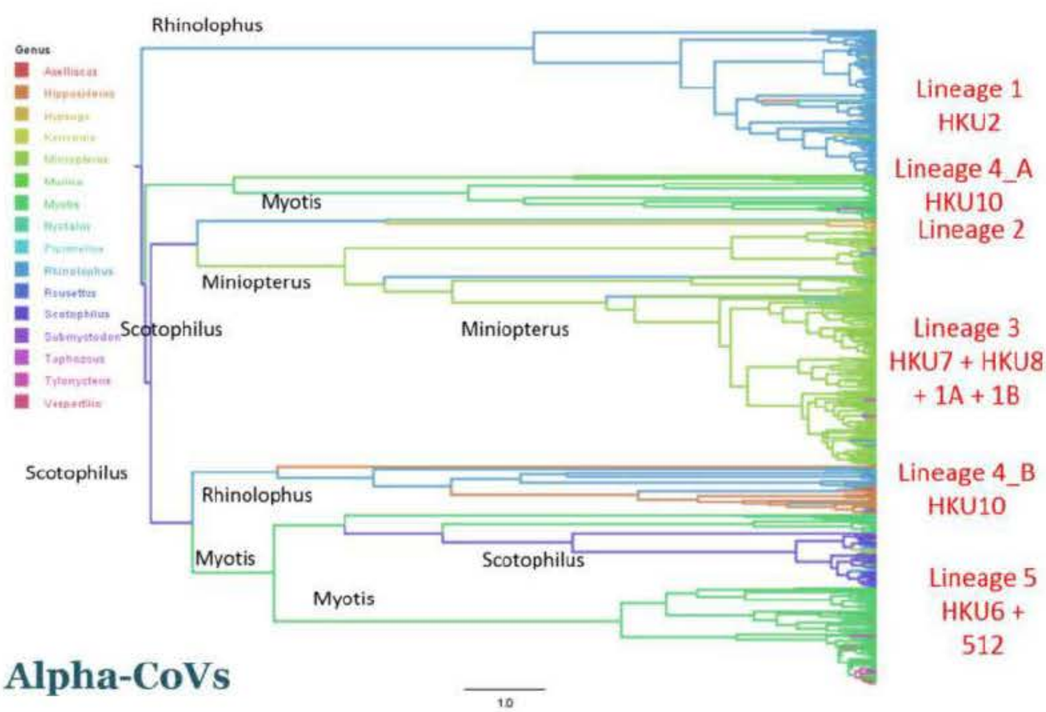


Figure 32. *Ancestral host reconstruction for Alpha-CoVs, at a host genus level.*

To better understand the geographic origins and extent of specific CoV clades, we also used discrete ancestral character state reconstruction in BEAST to reconstruct the ancestral location of each branch of the tree. We used SPREAD to visualize the tree in its geographic context and infer CoV spatial spread in China **(Fig. 33)**. These analyses allow us to identify the geographic areas that are likely sources of origin/diversity for this important group of viruses. The common ancestor of most Beta-CoVs lineages is located in Hong Kong and Guangdong. The common ancestor of most Alpha-CoV lineages was located in Yunnan province, and our results suggest they spread to other provinces from Yunnan.

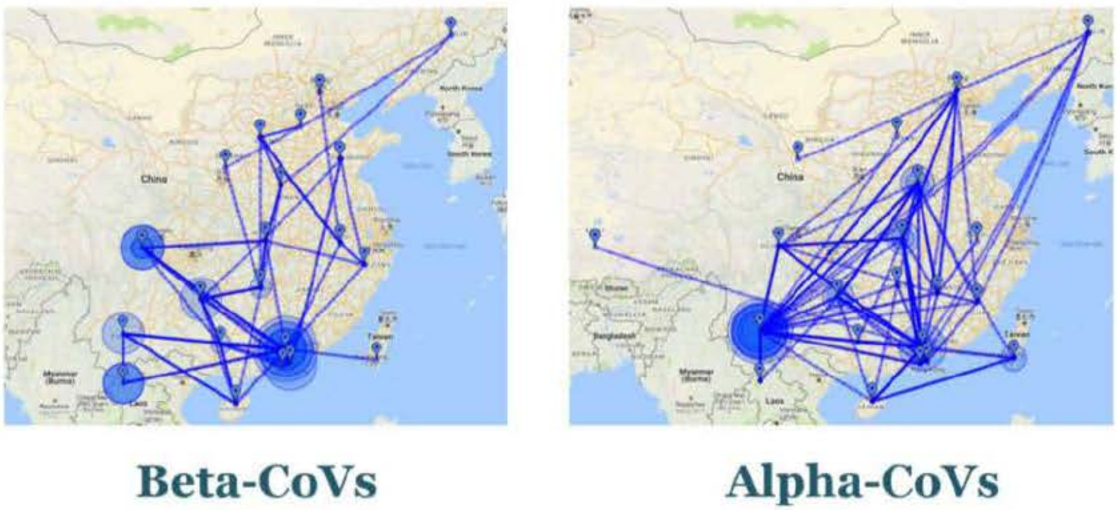


Figure 33. *Ancestral location reconstruction for Beta- and Alpha-CoVs. The bigger the circle is, the more ancestral the corresponding node is.*

<u>Specific Aim 3: Testing Predictions of CoV Inter-Species Transmission</u>

Identification of two novel MERS-related CoVs that use DPP4 receptor

Two novel MERSr-CoVs, BtCoV/Ii/GD/2013-845 and BtCoV/Ii/GD/2014-422, were identified from great evening bats (*Ia io*) in Guangdong province. Phylogenetic analysis of polyprotein 1 and the E, M, and N proteins suggests that the two novel strains are more closely related to MERS-CoV than to other lineage C Beta-CoVs. Their RdRp sequences are closely related to those of MERS-CoV and other MERSr-CoVs, with 94.4–97.0% aa identities. In contrast, they are divergent from MERS-CoV and other MERSr-CoVs in the spike protein, with only 58.9–64.7% aa identities. However, in the receptor-binding domain (RBD) of the spike protein, the two novel MERSr-CoVs are identical to MERS-CoV at six out of the 13 residues that directly interact with human DPP4 receptor, making them more similar to MERS-CoV than any other known lineage C BetaCoVs **(Fig. 34a)**. Protein–protein interaction assays demonstrated that the spike proteins of the novel MERSr-CoVs bind to both human and bat DPP4 **(Fig. 34b)**. Moreover, bat cells exogenously expressing human DPP4 support the entry of the retrovirus pseudotyped with BtCoV/Ii/GD/2014-422 spike, while the pseudovirus fails to enter cells that do not express DPP4. The results demonstrate that the spike protein of the newly identified MERSr-CoV recognizes the human DPP4 receptor.

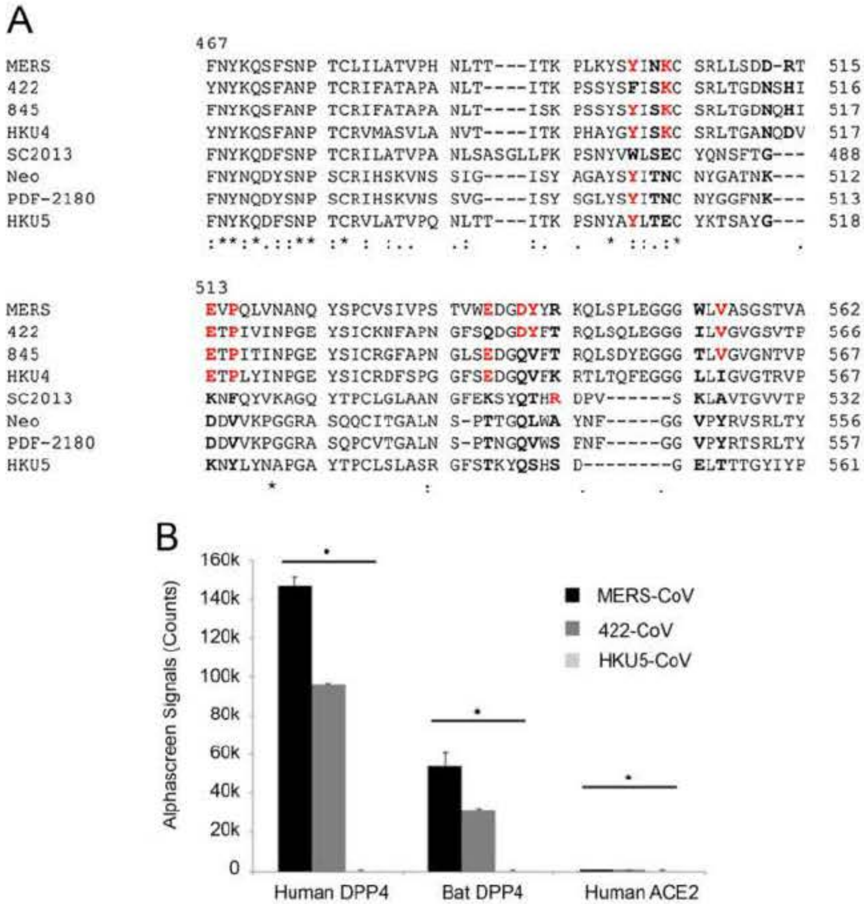


Figure 34. *BtCoV/li/GD/2014-422 RBD analysis (a) and DPP4-binding assay (b)*

In Vivo **Infection of Human ACE2 (hACE2) Expressing Mice with SARSr-CoV S Protein variants**

Using the reverse genetic methods we previously developed, infectious clones with the WIV1 backbone and the spike protein of SHC014, WIV16 and Rs4231, respectively, were constructed and recombinant viruses were successfully rescued. In Year 4, we performed preliminary *in vivo* infection of SARSr-CoVs on transgenic mice that express hACE2. Mice were infected with 10^5 pfu of full-length recombinant virus of WIV1 (rWIV1) and the three chimeric viruses with different spikes. Pathogenesis of the 4 SARSr-CoVs was then determined in a 2-week course. Mice challenged with rWIV1-SHC014S have experienced about 20% body weight loss by the 6th day post infection, while rWIV1 and rWIV-4231S produced less body weight loss. In the mice infected with rWIV1-WIV16S, no body weight loss was observed **(Fig. 35a)**. 2 and 4 days post infection, the viral load in lung tissues of mice challenged with rWIV1-SHC014S, rWIV1-WIV16S and rWIV1-Rs4231S reached more than 10^6 genome copies/g and were significantly higher than that in rWIV1-infected mice **(Fig. 35b)**. These results demonstrate varying pathogenicity of SARSr-CoVs with different spike proteins in humanized mice.

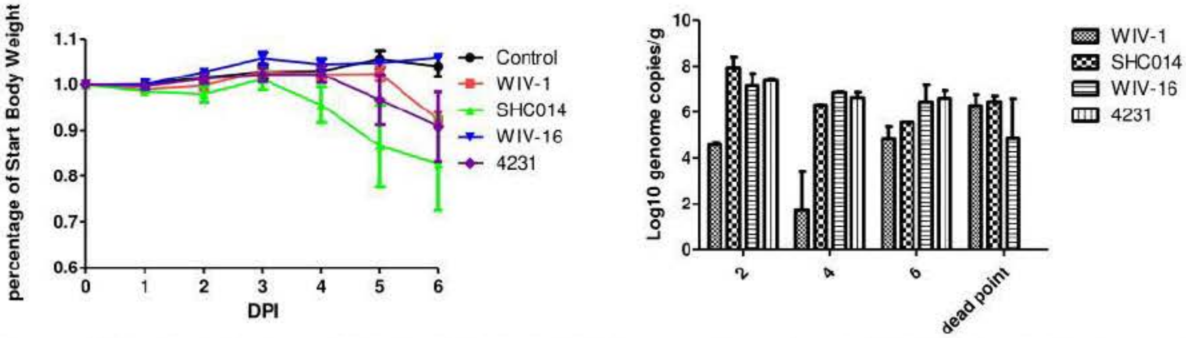


Figure 35. *In vivo infection of SARSr-CoVs in hACE2-expressing mice. (a, left) Body weight change after infection; (b, right) Viral load in lung tissues*

<u>Additional Year 4 Results for Specific Aim 3:</u>

Identification of a HKU2-related Coronavirus of Bat Origin that Caused Fatal Acute Diarrhea in Piglets

From October 2016, a series of fatal swine diarrhea disease outbreaks occurred in Guangdong province. By May 2017, it had resulted in death of 24,693 piglets across four farms. We identified a novel coronavirus as the etiological agent of the disease by metagenomic analysis, viral isolation and experimental infection, and named this “Swine Acute Diarrhea Syndrome coronavirus (SADS-CoV). During Year 4, we submitted and published a paper on this finding to *Nature* (Zhou *et al.*, 2018). The full-length genome of SADS-CoV shares 95% sequence identity to bat CoV HKU2. However, the S gene sequence identity is only 86%, suggesting that the previously reported HKU2-CoV is not the direct progenitor of SADS-CoV, but that they may have originated from a common ancestor.

Using a SADS-CoV specific qPCR assay based on its RdRp gene, SADS-related coronaviruses (SADSr-CoVs) were detected in rectal swabs of *Rhinolophus* bats collected from 2013 to 2016 in Guangdong. Full-length genome sequencing of 4 bat SADSr-CoVs revealed 96% to 98% overall genome sequence identity between SADSr-CoVs and SADS-CoV. Most importantly, the S protein of SADS-CoV shared more than 98% sequence identity with those of the two SADSr-CoVs (162149 and 141388), compared to 86% with HKU2-CoV (**Fig. *36a***). The phylogeny of S1 protein sequence showed strong co-evolutionary relationships with bat alphacoronavirus and their hosts, with swine SADS-CoV more closely related to SADSr-CoVs from *Rhinolophus affinis* than strains from *Rhinolophus sinicus* in which HKU2-CoV was found **(Fig. 36b)**. Analysis of the 33 SADS-CoV full genome sequences we were able to characterize from pigs suggests that viruses from the four farms may have been transmitted from their reservoir hosts independently. These findings highlight the importance of identifying coronavirus diversity and distribution in bats to mitigate future outbreaks that threaten livestock and public health.

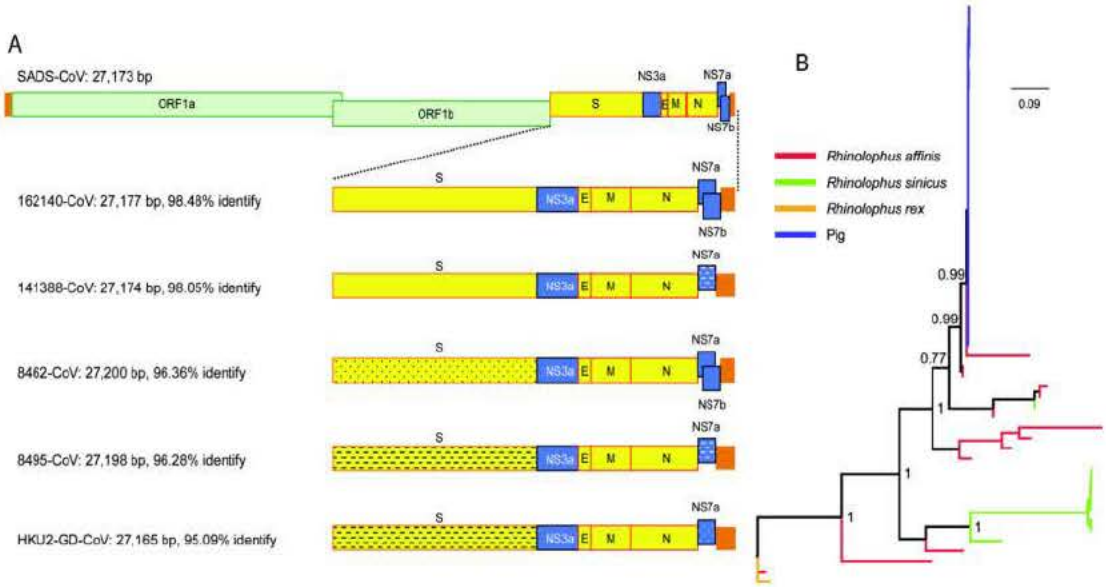
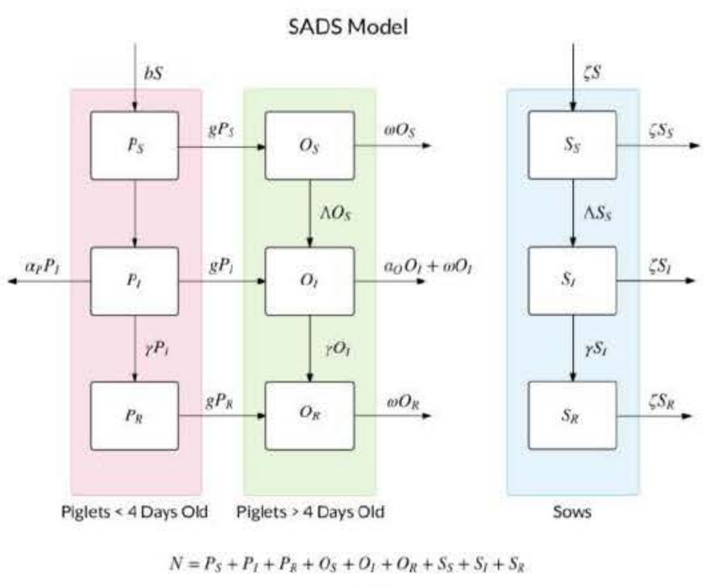


Figure 36. *Genome organization and comparison (a) and Phylogenetic analysis of S1 protein (b) of SADS-CoV and bat SADSr-CoVs*

Intra-Farm Transmission Model to Understand to Predict Future Transmission and Outbreak

To better understand amplification dynamics and assess the potential for future transmission resulting in large outbreaks, we developed an intra-farm, age-structured, stochastic transmission model for SADS-CoV **(Fig. 37)**. We developed multiple versions of this model to represent different hypotheses of disease transmission mechanisms and fit them to time-series data of reported deaths on multiple SADS-infected farms.



$$N = P_S + P_I + P_R + O_S + O_I + O_R + S_S + S_I + S_R$$

$$\Lambda = \beta \frac{(P_I+O_I+S_I)}{N^\rho}$$

Figure 37: *Schematic of intra-farm transmission mode.*

Our first model structure, which assumed equal mixing of animals across farms **(Fig. 38)** showed that age structure alone was insufficient to generate the temporal pattern of reported deaths on SADS-infected farms. Our second model structure **(Fig. 39)** represented individual barns on a farm as a series of pig-virus meta-populations. This structure was sufficient to re-create the dynamics of the series of rapid "mini-epidemics" that progressed in SADS-infected farms.

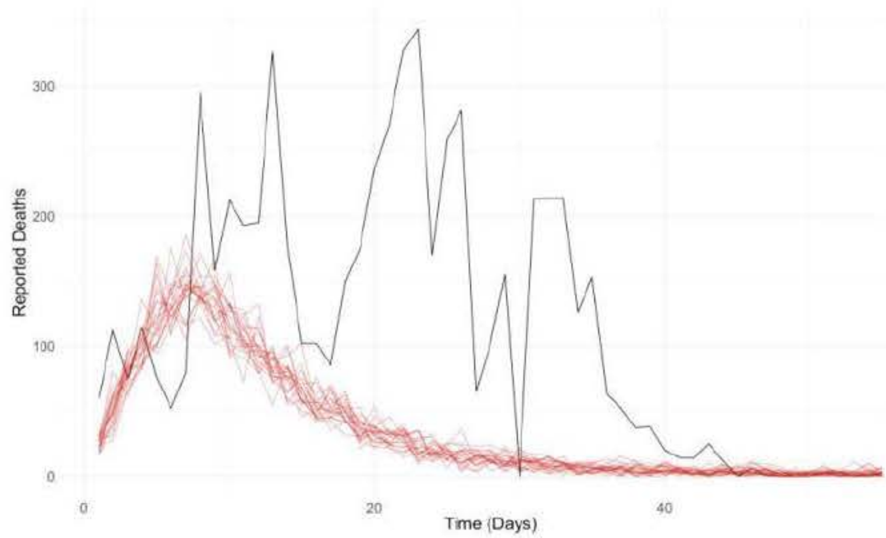


Figure 38: *Best-fit simulations (red) from an equal-mixing transmission model and actual reported death time series (black) on a SADS-infected farm.*

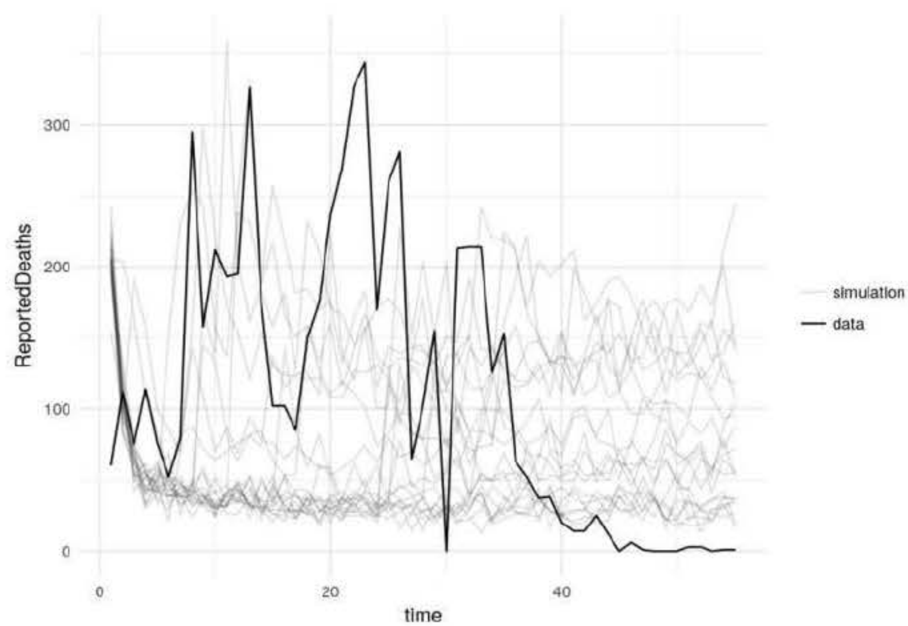


Figure 39: *Best-fit simulations (grey) from an metapopulation transmission model and actual reported death time series (black) on a SADS-infected farm.*

Specific Goals Not Meet

- The wild animal farm survey was piloted in early Y4, with data collected from seven wild animal farms, it was postponed due to the emergence of SADS-CoV where our group had focused on instead in Y4, but will be resumed in Y5 to continue collecting and analyzing data.
- The passive hospital surveillance has been piloted will continue in Year 4 to collect and test for CoVs.

B. 4 What opportunities for training and professional development has the project provided?

1. Conference and University lectures: We provided human subject research trainings to chief physicians and nurses at local clinics, staff from Yunnan Institute of Endemic Diseases Control and Prevention, students from Dali College and Wuhan University for both qualitative and quantitative research.

2. Agency and other briefing: Dr. Guangjian Zhu was invited by the Guangdong Institute of Applied Nature Resources, Guangdong Academy of Sciences to provide training to 8 field team members regarding biosafety and PPE use, bats and rodents sampling. Dr. Zhengli Shi participated in the US National Science Foundation-funded EcoHealthNet (grant to EcoHealth Alliance – Epstein PI) that provides research exchange opportunities to undergraduate and graduate-level students.

3. Public outreach: PI Daszak, and Co-investigators Shi, Epstein, and Olival presented the results of this project to the public via interviews with national central and local television, social media, newspaper and journals in China and the US.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Luo CM, Wang N, Yang XL, Liu HZ, Zhang W, Li B, Hu B, Peng C, Geng QB, Zhu GJ, Li F, Shi ZL. Discovery of Novel Bat Coronaviruses in South China That Use the Same Receptor as Middle East Respiratory Syndrome Coronavirus. Journal of virology. 2018 July 1;92(13). PubMed PMID: 29669833; PubMed Central PMCID: PMC6002729; DOI: 10.1128/JVI.00116-18.
Complete	Field HE. Evidence of Australian bat lyssavirus infection in diverse Australian bat taxa. Zoonoses and public health. 2018 September;65(6):742-748. PubMed PMID: 29785730; PubMed Central PMCID: PMC6249124; DOI: 10.1111/zph.12480.
Complete	Eskew EA, Olival KJ. De-urbanization and Zoonotic Disease Risk. EcoHealth. 2018 December;15(4):707-712. PubMed PMID: 30120670; PubMed Central PMCID: PMC6265062; DOI: 10.1007/s10393-018-1359-9.
Complete	Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nature reviews. Microbiology. 2019 March;17(3):181-192. PubMed PMID: 30531947; PubMed Central PMCID: PMC7097006; DOI: 10.1038/s41579-018-0118-9.
Complete	Li HY, Zhu GJ, Zhang YZ, Zhang LB, Hagan EA, Martinez S, Chmura AA, Francisco L, Tai H, Miller M, Daszak P. A qualitative study of zoonotic risk factors among rural communities in southern China. International health. 2020 February 12;12(2):77-85. PubMed PMID: 32040190; PubMed Central PMCID: PMC7017878; DOI: 10.1093/inthealth/ihaa001.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
[redacted] (b) (6)	Y	DASZAK, PETER	BS,PHD	PD/PI	[redacted]	[redacted]	(b) (4), (b) (6) [redacted]			NA
[redacted] (b) (6)	N	Chmura, Aleksei	BS,PHD	Non-Student Research Assistant	[redacted]	[redacted]	[redacted]			NA
[redacted] (b) (6)	N	Ross, Noam Martin	PhD	Co-Investigator	[redacted]	[redacted]	[redacted]			NA
[redacted] (b) (6)	Y	Olival, Kevin J.	PHD	Co-Investigator	[redacted]	[redacted]	[redacted]			NA
[redacted] (b) (6)	Y	Zhang, Shu-yi	PHD	Co-Investigator	[redacted]	[redacted]	[redacted]	East China Normal University	CHINA	NA
	N	ZHU, GUANGJIAN	PHD	Co-Investigator	[redacted]	[redacted]	[redacted]	East China Normal University	CHINA	NA
	N	GE, XINGYI	PHD	Co-Investigator	[redacted]	[redacted]	[redacted]	Wuhan Institute of Virology	CHINA	NA
	N	KE, CHANGWEN	PHD	Co-Investigator	[redacted]	[redacted]	[redacted]	Center for Disease Control and Prevention of Guangdong Province	CHINA	NA
	Y	ZHANG, YUNZHI	PHD	Co-Investigator	[redacted]	[redacted]	[redacted]	Yunnan Provincial Institute of Endemic Diseases Control & Prevention	CHINA	NA
[redacted] (b) (6)	N	EPSTEIN, JONATHAN H	MPH,DVM,BA,PHD	Co-Investigator	[redacted]	[redacted]	[redacted]			NA
[redacted] (b) (6)	N	SHI, ZHENGLI	PhD	Co-Investigator	[redacted]	[redacted]	[redacted]	Wuhan Institute of Virology	CHINA	NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Dollar Amount	Country
$201,422	CHINA

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Sub-Project ID:	Study ID	Study Title:	Delayed Onset	Clinical Trial	NCT	NIH-Defined Phase 3	ACT
	58010	Understanding the Risk of Bat Coronavirus Emergence-PROTOCOL-001	NO	NO		NO	

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

No

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: EcoHealth Alliance, Inc.	077090066	NY-010	460 West 34th Street 17th Floor New York NY 100012317
Wuhan Institute of Virology	529027474		Xiao Hong Shan, No. 44 Wuchang District Wuhan NONE
East China Normal University	420945495		3663 Zhongshan Beilu Shanghai NONE
ECOHEALTH ALLIANCE	077090066		ECOHEALTH ALLIANCE, INC. 460 W 34TH ST

			NEW YORK NY 100012320

G.9 FOREIGN COMPONENT

Organization Name: Wuhan Institute of Virology
Country: CHINA
Description of Foreign Component:
Principal Laboratory for all Research in China as per section G8 (above) and detailed in our Specific Aims

Organization Name: Wuhan School of Public Health
Country: CHINA
Description of Foreign Component:
Principal Coordinating Team for all project field work as per section G8 (above) and detailed in our Specific Aims

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

Section 1 - Basic Information (Study 58010)

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

1.1. Study Title *

Understanding the Risk of Bat Coronavirus Emergence-PROTOCOL-001

1.2. Is this study exempt from Federal Regulations *	❍ Yes	● No

1.3. Exemption Number ❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8

1.4. Clinical Trial Questionnaire *

1.4.a. Does the study involve human participants?	● Yes	❍ No
1.4.b. Are the participants prospectively assigned to an intervention?	❍ Yes	● No
1.4.c. Is the study designed to evaluate the effect of the intervention on the participants?	❍ Yes	● No
1.4.d. Is the effect that will be evaluated a health-related biomedical or behavioral outcome?	❍ Yes	● No

1.5. Provide the ClinicalTrials.gov Identifier (e.g. NCT87654321) for this trial, if applicable

Section 2 - Study Population Characteristics (Study 58010)

2.1. Conditions or Focus of Study

2.2. Eligibility Criteria

2.3. Age Limits	Min Age:	Max Age:

2.4. Inclusion of Women, Minorities, and Children

2.5. Recruitment and Retention Plan

2.6. Recruitment Status	Not yet recruiting

2.7. Study Timeline

Inclusion Enrollment Reports

IER ID#	Enrollment Location Type	Enrollment Location
IER 58010	Foreign	

Inclusion Enrollment Report 58010

Using an Existing Dataset or Resource* : ❍ Yes ● No

Enrollment Location Type* : ❍ Domestic ● Foreign

Enrollment Country(ies): CHN: CHINA

Enrollment Location(s):

Comments:

Planned

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	1230	1230	0	0	2460
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	0	0	0	0	0
White	0	0	0	0	0
More than One Race	0	0	0	0	0
Total	1230	1230	0	0	2460

Cumulative (Actual)

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/ Alaska Native	0	0	0	0	0	0	0	0	0	0
Asian	980	616	0	0	0	0	0	0	0	1596
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	0
Black or African American	0	0	0	0	0	0	0	0	0	0
White	0	0	0	0	0	0	0	0	0	0
More than One Race	0	0	0	0	0	0	0	0	0	0
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	0
Total	980	616	0	0	0	0	0	0	0	1596

Section 3 - Protection and Monitoring Plans (Study 58010)

3.1. Protection of Human Subjects

3.2. Is this a multi-site study that will use the same protocol to conduct non-exempt human subjects research at more than one domestic site?	❍ Yes	❍ No	❍ N/A

If yes, describe the single IRB plan

3.3. Data and Safety Monitoring Plan

3.4. Will a Data and Safety Monitoring Board be appointed for this study?	❍ Yes	❍ No

3.5. Overall structure of the study team

Section 4 - Protocol Synopsis (Study 58010)

4.1. Brief Summary

4.2. Study Design

4.2.a. Narrative Study Description

4.2.b. Primary Purpose

4.2.c. Interventions

Type	Name	Description

4.2.d. Study Phase

Is this an NIH-defined Phase III Clinical Trial? ❍ Yes ● No

4.2.e. Intervention Model

4.2.f. Masking ❍ Yes ❍ No

❑ Participant ❑ Care Provider ❑ Investigator ❑ Outcomes Assessor

4.2.g. Allocation

4.3. Outcome Measures

Type	Name	Time Frame	Brief Description

4.4. Statistical Design and Power

4.5. Subject Participation Duration

4.6. Will the study use an FDA-regulated intervention? ❍ Yes ❍ No

4.6.a. If yes, describe the availability of Investigational Product (IP) and Investigational New Drug (IND)/ Investigational Device Exemption (IDE) status

4.7. Dissemination Plan

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 07/13/2020

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 2R01AI110964-06 REVISED
FAIN: R01AI110964

Principal Investigator(s):
PETER DASZAK, PHD

Project Title: Understanding the Risk of Bat Coronavirus Emergence

Dr. Daszak, Peter
PD/PI
460 West 34th Street
Suite 1701
New York, NY 100012320

Award e-mailed to: [redacted] (b) (6)

Period Of Performance:
Budget Period: 07/24/2019 – 06/30/2021
Project Period: 06/01/2014 – 06/30/2025

Dear Business Official:

The National Institutes of Health hereby revises this award to reflect an increase in the amount of $369,819 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to ECOHEALTH ALLIANCE, INC. in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI110964. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Emily Linde
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 2R01AI110964-06 REVISED

Award Calculation (U.S. Dollars)

Salaries and Wages	$170,325
Fringe Benefits	$53,654
Personnel Costs (Subtotal)	$223,979
Consultant Services	$49,809
Materials & Supplies	$20,170
Travel	$15,045
Subawards/Consortium/Contractual Costs	$229,923
Federal Direct Costs	$538,926
Federal F&A Costs	$123,054
Approved Budget	$661,980
Total Amount of Federal Funds Obligated (Federal Share)	$661,980
TOTAL FEDERAL AWARD AMOUNT	$661,980
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$369,819

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
6	$661,980	$661,980
7	$637,980	$637,980
8	$637,980	$637,980
9	$637,980	$637,980
10	$637,980	$637,980

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name: Allergy and Infectious Diseases Research
CFDA Number: 93.855
EIN: 1311726494A1
Document Number: RAI110964B
PMS Account Type: P (Subaccount)
Fiscal Year: 2019

IC	CAN	2019	2021	2022	2023	2024
AI	8472364	$661,980	$637,980	$637,980	$637,980	$637,980

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M51C B / **OC**: 41022 / **Released**: (b) (6) 07/13/2020
Award Processed: 07/15/2020 12:00:48 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 2R01AI110964-06 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 2R01AI110964-06 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.

c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI110964. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 2R01AI110964-06 REVISED

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

REVISED AWARD: Pursuant to the letter to EcoHealth Alliance, Inc. dated July 8, 2020, this award has been reinstated; however, all activities are suspended until such time as these concerns in the letter have been addressed to NIH's satisfaction.

Supersedes previous Notice of Award dated 04/27/2020. All other terms and conditions still apply to this award.

REVISED AWARD: This award is revised to adjust the budget in accordance with the letter from Aleksei Chmura/ECOHealth Alliance.

Supersedes previous Notice of Award dated **07/24/2019**.

****

This Notice of Award (NoA) includes funds for activity with **The University of North Carolina at Chapel Hill** in the amount of **$77,750** (**$50,000** direct costs + **$27,750**F&A costs).

This Notice of Award (NoA) includes funds for activity with **Wuhan Institute of Virology** in the amount of **$76,301** (**$70,649** direct costs + **$5,652** F&A costs).

This Notice of Award (NoA) includes funds for activity with **Institute of Pathogen Biology** in the amount of **$75,600** (**$70,000** direct costs + **$5,600** F&A costs).

****

The Research Performance Progress Report (RPPR), Section G.9 (Foreign component), includes reporting requirements for all research performed outside of the United States. Research conducted at the following site(s) must be reported in your RPPR:

Wuhan Institute of Virology, CHINA

Institute of Pathogen Biology, CHINA

East China Normal University, CHINA

Duke-NUS Medical School, SINGAPORE

****

This award reflects current Federal policies regarding Facilities & Administrative (F&A) Costs for foreign grantees including foreign sub-awardees, and domestic awards with foreign sub-awardees. Please see: Chapter 16 Grants to Foreign Organizations, International Organizations, and Domestic Grants with Foreign Components, Section 16.6 "Allowable and Unallowable Cost" of the NIH Grants Policy.

****

This award may include collaborations with and/or between foreign organizations. Please be advised that short term travel visa expenses are an allowable expense on this grant, if justified as critical and necessary for the conduct of the project.

****

The budget period anniversary start date for future year(s) will be **July** 1.

****

Dissemination of study data will be in accord with the Recipient's accepted genomic data sharing plan as stated in the page(s) **203** of the application. Failure to adhere to the sharing plan as mutually agreed upon by the Recipient and the NIAID may result in Enforcement Actions as described in the NIH Grants Policy Statement.

****

This award is subject to the Clinical Terms of Award referenced in the NIH Guide for Grants and Contracts, July 8, 2002, NOT AI-02-032. These terms and conditions are hereby incorporated by reference, and can be accessed via the following World Wide Web address: https://www.niaid.nih.gov/grants-contracts/niaid-clinical-terms-award All submissions required by the NIAID Clinical Terms of Award must be forwarded electronically or by mail to the responsible NIAID Program Official identified on this Notice of Award.

****

Awardees who conduct research involving Select Agents (see 42 CFR 73 for the Select Agent list; and 7 CFR 331 and 9 CFR 121 for the relevant animal and plant pathogens at http://www.selectagents.gov/Regulations.html) must complete registration with CDC (or APHIS, depending on the agent) before using NIH funds. No funds can be used for research involving Select Agents if the final registration certificate is denied.

Prior to conducting a restricted experiment with a Select Agent or Toxin, awardees must notify the NIAID and must request and receive approval from CDC or APHIS.

****

Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

> Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.
>
> If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- o A list of the new and/or additional Agent(s) that will be studied;
- o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Shaun W Gratton
Email: [redacted] (b) (6) **Phone**: [redacted] (b) (6)

Program Official: Erik J. Stemmy
Email: [redacted] (b) (6) **Phone**: [redacted] (b) (6)

SPREADSHEET SUMMARY
GRANT NUMBER: 2R01AI110964-06 REVISED

INSTITUTION: ECOHEALTH ALLIANCE, INC.

Budget	Year 6	Year 7	Year 8	Year 9	Year 10
Salaries and Wages	$170,325	$170,123	$170,123	$170,123	$170,123
Fringe Benefits	$53,654	$53,590	$53,590	$53,590	$53,590
Personnel Costs (Subtotal)	$223,979	$223,713	$223,713	$223,713	$223,713
Consultant Services	$49,809	$49,750	$49,750	$49,750	$49,750
Materials & Supplies	$20,170	$14,850	$14,850	$14,850	$14,850
Travel	$15,045	$15,027	$15,027	$15,027	$15,027
Subawards/Consortium/Contractual Costs	$229,923	$229,651	$229,651	$229,651	$229,651
Publication Costs		$6,000	$6,000	$6,000	$6,000
TOTAL FEDERAL DC	$538,926	$538,991	$538,991	$538,991	$538,991
TOTAL FEDERAL F&A	$123,054	$98,989	$98,989	$98,989	$98,989
TOTAL COST	$661,980	$637,980	$637,980	$637,980	$637,980

Facilities and Administrative Costs	Year 6	Year 7	Year 8	Year 9	Year 10
F&A Cost Rate 1	32%	32%	32%	32%	32%
F&A Cost Base 1	$384,547	$309,340	$309,340	$309,340	$309,340
F&A Costs 1	$123,054	$98,989	$98,989	$98,989	$98,989

PI: **DASZAK, PETER**	Title: Understanding the Risk of Bat Coronavirus Emergence	
Received: 11/05/2018	FOA: PA18-484 Clinical Trial:Not Allowed	Council: 05/2019
Competition ID: FORMS-E	FOA Title: NIH Research Project Grant (Parent R01 Clinical Trial Not Allowed)	
2 R01 AI110964-06	Dual:	Accession Number: 4237214
IPF: 4415701	Organization: ECOHEALTH ALLIANCE, INC.	
Former Number:	Department:	
IRG/SRG: CRFS	AIDS: N	Expedited: N
<u>Subtotal Direct Costs</u> <u>(excludes consortium F&A)</u> Year 6: 515,358 Year 7: 515,358 Year 8: 515,358 Year 9: 515,358 Year 10: 515,358	Animals: Y Humans: Y Clinical Trial: N Current HS Code: (b) (4) HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
PETER DASZAK	ECOHEALTH ALLIANCE, INC.	PD/PI
Zheng Li Shi	Wuhan Institute of Virology	Co-Investigator
Kevin Olival	EcoHealth Alliance	Co-Investigator
Ralph Baric	University of North Carolina	Co-Investigator
Noam Ross	EcoHealth Alliance	Co-Investigator
Alice Latinne	EcoHealth Alliance	Other (Specify)-Research Scientist
HongYing Li	EcoHealth Alliance	Other (Specify)-Research Scientist
Leilani Francisco	EcoHealth Alliance	Co-Investigator
Amy Sims	University of North Carolina at Chapel Hill	Co-Investigator
Emily Hagan	EcoHealth Alliance	Other (Specify)-Research Scientist
Guangjian Zhu	East China Normal University	Co-Investigator
Linfa Wang	Duke-NUS Medical School	Co-Investigator
Lili Ren	Institute of Pathogen Biology	Co-Investigator
Li Guo	Institute of Pathogen Biology	Co-Investigator
Peng Zhou	Wuhan Institute of Virology	Co-Investigator
Ben Hu	Wuhan Institute of Virology	Co-Investigator
Aleksei Chmura	EcoHealth Alliance	Other (Specify)-Research Scientist

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier
AI110964

b. Agency Routing Number

2. DATE SUBMITTED	**Application Identifier**	**c. Previous Grants.gov Tracking Number**

5. APPLICANT INFORMATION **Organizational DUNS*:** 0770900660000

Legal Name*: ECOHEALTH ALLIANCE, INC.
Department:
Division:
Street1*: ECOHEALTH ALLIANCE, INC.
Street2: 460 W 34TH ST
City*: NEW YORK
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 100012320

Person to be contacted on matters involving this application
Prefix: Dr. First Name*: Peter Middle Name: Last Name*: Daszak Suffix:
Position/Title: PD/PI
Street1*: 460 West 34th Street
Street2: Suite 1701
City*: New York
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 100012320
Phone Number*: [redacted] (b) (6) Fax Number: 2123804465 Email: [redacted] (b) (6)

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 311726494

7. TYPE OF APPLICANT* M: Nonprofit with 501C3 IRS Status (Other than Institution of Higher Education)

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*
❍ New ❍ Resubmission
● Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Understanding the Risk of Bat Coronavirus Emergence

12. PROPOSED PROJECT

Start Date*	Ending Date*
06/01/2019	05/31/2024

13. CONGRESSIONAL DISTRICTS OF APPLICANT
NY-010

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE **Page 2**

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: PETER Middle Name: Last Name*: DASZAK Suffix:
Position/Title: President
Organization Name*: ECOHEALTH ALLIANCE, INC.
Department:
Division:
Street1*: 460 West 34th Street
Street2: Suite 1701
City*: New York
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 100012317
Phone Number*: [redacted] (b) (6) Fax Number: +12123804465 Email*: [redacted] (b) (6)

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$3,586,760.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$3,586,760.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Dr. First Name*: Aleksei Middle Name: Last Name*: Chmura Suffix:
Position/Title*: Authorized Organizational Representative
Organization Name*: EcoHealth Alliance, Inc.
Department:
Division:
Street1*: 460 West 34th Street
Street2: Suite 1701
City*: New York
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 100012320
Phone Number*: [redacted] (b) (6) Fax Number: 2123804465 Email*: [redacted] (b) (6)

Signature of Authorized Representative*	Date Signed*
Aleksei Chmura	11/05/2018

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:NIAID_COV_2018_Cover_Letter_Final.pdf

424 R&R and PHS-398 Specific
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OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	ECOHEALTH ALLIANCE, INC.
Duns Number:	0770900660000
Street1*:	ECOHEALTH ALLIANCE, INC.
Street2:	460 W 34TH ST
City*:	NEW YORK
County:	
State*:	NY: New York
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	100012320
Project/Performance Site Congressional District*:	NY-010

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	University of North Carolina at Chapel Hill
DUNS Number:	6081952770000
Street1*:	McGavran-Greenberg Hall
Street2:	Campus Box 7435
City*:	Chapel Hill
County:	
State*:	NC: North Carolina
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	275997435
Project/Performance Site Congressional District*:	NC-004

Project/Performance Site Location 2

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Wuhan Institute of Virology
DUNS Number: 5290274740000
Street1*: Xiao Hong SHan, No. 44
Street2: Wuchang District
City*: Wuhan
County:
State*:
Province:
Country*: CHN: CHINA
Zip / Postal Code*: 430071
Project/Performance Site Congressional District*: 00-000

Project/Performance Site Location 3

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Institute of Pathogen Biology
DUNS Number: 5281563570000
Street1*: Dong Dan San Tiao, No. 9
Street2: Dongcheng District
City*: Beijing
County:
State*:
Province:
Country*: CHN: CHINA
Zip / Postal Code*: 100730
Project/Performance Site Congressional District*: 00-000

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ❍ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ● No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ● **Yes** ❍ **No**

IRB Approval Date: 03-15-2019

Human Subject Assurance Number None

2. Are Vertebrate Animals Used?* ● **Yes** ❍ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ❍ No

IACUC Approval Date: 03-15-2019

Animal Welfare Assurance Number None

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ● **Yes** ❍ **No**

6.a. If yes, identify countries: China

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	NIAID_COV_2019_PROJECT_SUMMARY_final.pdf
8. Project Narrative*	NIAID_COV_2019_NARRATIVE_Final.pdf
9. Bibliography & References Cited	NIAID_COV_2019_REFERENCES.pdf
10.Facilities & Other Resources	NIAID_COV_2019_FACILITIES_v01_PD.pdf
11.Equipment	NIAID_COV_2019_EQUIPMENT_v01.pdf

Project Summary: Understanding the Risk of Bat Coronavirus Emergence
Novel zoonotic, bat-origin CoVs are a significant threat to global health and food security, as the cause of SARS in China in 2002, the ongoing outbreak of MERS, and of a newly emerged Swine Acute Diarrhea Syndrome in China. In a previous R01 we found that bats in southern China harbor an extraordinary diversity of SARSr-CoVs, some of which can use human ACE2 to enter cells, infect humanized mouse models causing SARS-like illness, and evade available therapies or vaccines. We found that people living close to bat habitats are the primary risk groups for spillover, that at one site diverse SARSr-CoVs exist that contain every genetic element of the SARS-CoV genome, and identified serological evidence of human exposure among people living nearby. These findings have led to **18 published peer-reviewed papers, including two papers in *Nature*, and a review in *Cell*.** Yet salient questions remain on the origin, diversity, capacity to cause illness, and risk of spillover of these viruses. In this R01 renewal we will address these issues through 3 specific aims:
Aim 1. Characterize the diversity and distribution of high spillover-risk SARSr-CoVs in bats in southern China. We will use phylogeographic and viral discovery curve analyses to target additional bat sample collection and molecular CoV screening to fill in gaps in our previous sampling and fully characterize natural SARSr-CoV diversity in southern China. We will sequence receptor binding domains (spike proteins) to identify viruses with the highest potential for spillover which we will include in our experimental investigations (Aim 3).
Aim 2. Community, and clinic-based syndromic, surveillance to capture SARSr-CoV spillover, routes of exposure and potential public health consequences. We will conduct biological-behavioral surveillance in high-risk populations, with known bat contact, in community and clinical settings to 1) identify risk factors for serological and PCR evidence of bat SARSr-CoVs; & 2) assess possible health effects of SARSr-CoVs infection in people. We will analyze bat-CoV serology against human-wildlife contact and exposure data to quantify risk factors and health impacts of SARSr-CoV spillover.
Aim 3. *In vitro* and *in vivo* characterization of SARSr-CoV spillover risk, coupled with spatial and phylogenetic analyses to identify the regions and viruses of public health concern. We will use S protein sequence data, infectious clone technology, *in vitro* and *in vivo* infection experiments and analysis of receptor binding to test the hypothesis that % divergence thresholds in S protein sequences predict spillover potential. We will combine these data with bat host distribution, viral diversity and phylogeny, human survey of risk behaviors and illness, and serology to identify SARSr-CoV spillover risk hotspots across southern China. Together these data and analyses will be critical for the future development of public health interventions and enhanced surveillance to prevent the re-emergence of SARS or the emergence of a novel SARSr-CoV.

Renewal: Understanding the Risk of Bat Coronavirus Emergence

Project Narrative

Most emerging human viruses come from wildlife, and these represent a significant threat to public health and biosecurity in the US and globally, as was demonstrated by the SARS coronavirus pandemic of 2002-03. This project seeks to understand what factors allow coronaviruses, including close relatives to SARS, to evolve and jump into the human population by studying viral diversity in their animal reservoirs (bats), surveying people that live in high-risk communities in China for evidence of bat-coronavirus infection, and conducting laboratory experiments to analyze and predict which newly-discovered viruses pose the greatest threat to human health.

Facilities, Equipment, and Other Resources

<u>EcoHealth Alliance, New York, USA (Drs. Daszak, Olival, Francisco, Ross)</u>

EcoHealth Alliance is a New York-based 501(c) 3 non-profit institution that conducts scientific research on emerging zoonoses and global health capacity building. EcoHealth Alliance New York headquarters has [redacted] (b) (4) square feet of office space including a meeting room and basic laboratory – freezer storage and light microscopy. The scientific staff (34 core scientists, 100+ field staff) is supported by a core admin staff of 18 who are available for work on this project and funded through private donor and federal support. EcoHealth Alliance does not support diagnostic facilities at its core headquarters and works in partnership with a network of leading diagnostic labs both in the USA and around the world.

EcoHealth Alliance is equipped with fiber optic Internet access and video conferencing facilities to facilitate easy communication between collaborators. EcoHealth Alliance employees have around-the-clock access to servers, VPNs, encryption software, IT support, and all necessary software including Git and Github (Hosted software revision/audit service), Sublime and Vim text editors, Vagrant and Oracle Virtualbox virtual machines, Google Apps (Hosted email and collaboration web based software), Ansible (Server provisioning software framework), Python, NodeJS, and R programming languages, Meteor (Javascript framework), Bash shell scripts, Jenkins (Continuous Integration server), Microsoft Office and Adobe CS6 running on both Apple Mac OS X, Ubuntu linux, and Windows Operating Systems. EcoHealth Alliance has a dedicated quad-core Linux server and another dedicated dual quad-core Mac Pro Server - each with 4TB hard drives. Either server individually or in combination may be used for intensive computational modeling and/or database processing by all the grantees. Access to the cloud and supercomputing services (Amazon) is provided by core funding to EcoHealth Alliance.

EcoHealth Alliance is the headquarters of a global network of over 70 partners that provides exceptional leverage for the core scientists. This network includes staff from: academic institutions at leading national universities; intergovernmental agencies (WHO, OIE, FAO, DIVERSITAS, IUCN); infectious disease surveillance laboratories including BSL-3 and -4 laboratories; national government agency offices and labs; locally-based wildlife conservation organizations in Asia, Africa and Latin America. EcoHealth Alliance is the headquarters of: The Consortium for Conservation Medicine (CCM); the journal *EcoHealth*; an NSF Research Coordination Network (EcoHealthNET); the IUCN Wildlife Health Specialist Group; and the OIE Wildlife Health Network. EcoHealth Alliance is a voting member of the IUCN and a partner in Columbia University's Earth Institute Center for Environmental Sustainability (EICES) and all senior scientific staff members are Adjunct Faculty at Columbia University's Department of Ecology, Evolution, and Environmental Biology or at the Mailman School of Public Health.

<u>Institute of Pathogen Biology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China (Drs. Ren, Guo)</u>

The Institute of Pathogen Biology (IPB) is a key (flagship) institute within the Chinese Academy of Medical Sciences & Peking Union Medical College. IPB's mission is to conduct high quality research in basic and applied biology of critically important human pathogens. The ultimate goal is to conduct research and develop technology for better diagnosis, treatment, and prevention of infectious diseases. IPB specializes in multi-disciplinary approaches to pathogen research and technological development focused on improving China's capability to diagnose, treat and prevent infectious diseases.

Human Resources. The department involved in this project consists of 30 staff members: 10 with a clinical medicine background, 12 biological research scientists, 3 bioinformaticists, 3 animal technicians, and 2 biochemists working on protein expression and purification.

Lab Facilities. The IPB includes the Ministry of Health Key Laboratory of Systems Biology of Pathogens, Christophe Merieux Laboratory, the AIDS Research Center, laboratories focused on Bacteriology, Virology, Mycology, Parasitology, and the Epidemiological Information Analysis Department. The institute has established platforms in metagenomics, transcriptome, morphology, molecular biology, and immunology. All of these are funded centrally and available to conduct the research proposed in the current R01.

BSL2 Facility. The institute has three laboratories of [redacted] (b) (4) equipped as BSL2 space for virology, immunology and clinical sample preparation. Equipment includes an Illumina Hiseq 2500, Miseq and BGI 500, gel electrophoresis, power supplies, thermal cyclers, a programmable heat block, heat blocks, water baths, CO_2 incubators (2), several -70°C freezers, one -140°C freezer, refrigerators, DNA documentation system, DNA sequencing and computer assisted sequence analysis programs, several microfuges, Nikon and Zeiss microscopes with photographic and fluorescent capabilities, several class 2 environmental hoods, refrigerated water baths, real time thermocyclers, and spectrophotometers. The laboratory has an ELISA plate reader, an illuminometer, ELISA plate washer, spectrophotometers, and other equipment that is routinely used in characterizing antibody-protein interactions.

BSL 3 Facility. The institute shares an additional [redacted] (b) (4) of BSL3 facilities equipped with sterile hoods (BSCIIA), CO_2 incubators, -70C freezer, an inverted Nikon fluorescent microscope, and equipment for virus isolation and culture, and molecular genetics research.

__University of North Carolina at Chapel Hill, USA (Baric and Sims)__

The Department of Epidemiology is an internationally recognized leader in epidemiologic research and training. The department offers research training in most specialized areas including cancer, cardiovascular diseases, environmental and occupational health, health services/clinical epidemiology, reproductive health and infectious diseases. The department's current faculty consists of 51 regular full-time faculty and 151 adjunct faculty members. The department has 218 graduate students enrolled, including 20 in the MPH program, 5 in the MSPH program, 20 in the MSCR program and 173 in the Ph.D. program. The Department of Epidemiology is headquartered in the four-story McGavran-Greenberg Building. The epidemiology administrative and office space occupies [redacted] (b) (4) square feet and provides additional classroom space. Most of the department's research staff occupies a research annex consisting of approximately [redacted] (b) (4) square feet of contiguous rental space in a commercial office building.

Dr. Baric has three laboratories of [redacted] (b) (4) square feet each equipped as BL2 space for molecular biology, virology, immunology and recombinant DNA techniques, as laid out in the current R01 proposal. Equipment is available for gel electrophoresis, PCR, and BSL2 sample storage and handling facilities. It includes a DNA documentation system, DNA sequencing and computer assisted sequence analysis programs, several microfuges, a microscopy suite, 10+ IBM and Apple Pentium II/III computers with accompanying software, three thermocyclers, a fume hood, Nuclisens reader, hybridization oven, real time thermocyclers, three fluorescent inverted scopes with computer software (Olympus IX51), and a spectrophotometer. A Roche Light Cycler 480II is available for real time measurements. The laboratory has an ELISA plate reader, an illuminometer, 200 cages for animal maintenance and breeding in Seal-Safe housing, Bio Rad low pressure chromatography system, ELISA plate washer, spectrophotometers, and other equipment that is routinely used in characterizing antibody-protein interactions.

The Baric laboratory contains an additional [redacted] (b) (4) square feet of newly renovated BSL3 facilities with enhanced features including shower in/shower out facility; dual anteroom access; Hepa filtered exhaust; redundant exhaust fans; card key access; an alarm system to Public Health/Campus Police; laboratory controlled combination lock; and Techniplast Sealsafe™ Hepa filtered animal housing for 300+ rodents. PAPR and tyvek suits are worn at all times in the BSL3 facility. The BL3 facilities are in an adjacent and attached building [redacted] (b) (4) or in [redacted] (b) (4), the latter space is directly adjacent to Dr. Baric's BSL2 laboratory resources. Each facility is equipped with sterile hoods (BSCIIA), four CO_2 incubators, gel electrophoresis equipment, thermal cyclers and power supplies, and related equipment necessary for virus cultivation and molecular genetic research. The facilities each house a -70°C freezer, an inverted Nikon fluorescent microscope with a digital camera, an ELISA plate reader and illuminometer. Both facilities contain rodent-sized Seal-Safe systems (~192 cages) for maintaining animals in a Hepa-filtered Air in/out environment, exhausted into the BSL3 Hepa-filtered exhaust system. An 8 chamber Buxco plethysmography system that allows for repetitive, noninvasive measures of the number of breaths, tidal volume, airway responsiveness, enhanced pause, and respiratory gases from live control and infected mice in a contained system is housed in the main BSL3 laboratory in [redacted] (b) (4).

The Department of Epidemiology provides cold-room, autoclave, centralized dishwashing and a darkroom with an automated developer. The campus has central facilities for DNA oligonucleotide synthesis, histopathology, DNA sequencing, EM, light and confocal microscopy, automated PCR genotyping and Taqman facilities, and Fluorescent activated cell sorter facilities (FAC). As a member of the Department of Microbiology and Immunology and UNC Cancer center, Dr. Baric and his team have access to these facilities at a discounted cost. The University provides a variety of core services including: sequencing and deep sequencing cores, genomics cores, oligonucleotide synthesis cores, hybridoma cores, transgenic cores, structural biology cores, etc. typical of any world class research institution. Campus wide core facilities are available for oligonucleotide synthesis, Sanger and 454 sequencing, RNAseq, pathology and histology services, and Flow Cytometry. Approximately, 40,000 cages are available for CC RIX production in the [redacted] (b) (4) on UNC Campus.

Wuhan Institute of Virology, Chinese Academy of Sciences, Hubei, China (Shi, Zhou, and Hu)

The Wuhan Institute of Virology (WIV), Chinese Academy of Sciences (CAS) is the only institute specializing in virology, viral pathology and virus technology among 19 other biological and biomedical research institutes in CAS. WIV is China's premier institute for virologic research. It consists of three research departments and one center: the Departments of Molecular Virology, of Bio-control, of Analytical Biochemistry and Biotechnology, and the Virus Resource and Bioinformation Center. It contains the Key Laboratory of Molecular Virology of CAS, the Joint-laboratory of Invertebrate Virology, an HIV Pre-screening Lab and the Hubei Engineering and Technology Research Center for Viral Diseases. The institute is further divided into 14 research groups, one of which (the Emerging Virus Laboratory) is headed by Dr. Zhengli Shi. The supporting system of the institute consists of an analytical equipment center, an experimental animal center, the editorial office of *Virologica Sinica* and a computer network center. The virus resource and bio-information center of China contains the largest virus bank in Asia, curating around 800 viral strains.

The Wuhan Institute of Virology is a World Health Organization collaborating center. It also has partnerships, research collaborations and contracts with universities and research institutes in more than 30 counties and regions including a long-time (>15 years) partnership with EcoHealth Alliance. There are 14 professors, 36 associated professors, and 47 assistant professors conducting research on virology and five of these have been awarded honors in the “Hundred Talents Project”. In 2013, the first BSL-4 lab in China was opened at this Institute in a bespoke facility which was designed with the assistance of the US CDC and L'Institut Pasteur of France.

The WIV Emerging Virus Laboratory, headed by Dr. Shi, was set up to carry out exactly the sort of experimental activities on emerging viruses listed in the current R01 proposal. This lab possesses all necessary facilities for molecular biology and virology including a bank of -80˚C freezers, PCR machines, gel electrophoresis and imaging systems, biosafety cabinets, super-clean benches, and cell culture rooms. A Core Facility Center was established at WIV to provide technological services to faculty, students, and visiting researchers. Core Facility Center equipment includes: a transmission electron microscope, ultracentrifugation machines, small animal *in vivo* imaging systems, confocal laser scanning microscopes, flow cytometry, a real-time qPCR system, and a high-throughput sequencing and analyzing system. In addition, WIV owns a complete biosafety research platform, which consists of the first national BSL-4 laboratory in China, and a cluster of BSL-3 and BSL-2 labs.

Equipment

EcoHealth Alliance (Daszak, Francisco, Olival, Ross)

EcoHealth Alliance is equipped with fiber optic Internet access and video conferencing facilities to facilitate easy communication between collaborators. EcoHealth Alliance employees have around the clock access to servers, VPNs, encryption software, IT support, and all necessary software including Git and Github (Hosted software revision/audit service), Sublime and Vim text editors, Vagrant and Oracle Virtualbox virtual machines, Google Apps (Hosted email and collaboration web based software), Ansible (Server provisioning software framework), Python, NodeJS, and R programming languages, Meteor (Javascript framework), Bash shell scripts, Jenkins (Continuous Integration server), Microsoft Office and Adobe CS6 running on both Apple Mac OS X, Ubuntu linux, and Windows Operating Systems. Additionally, EcoHealth Alliance has a dedicated quad-core Linux server and another dedicated dual quad-core Mac Pro Server - each with 4TB hard drives. Either server individually or in combination may be used for intensive computational modeling and/or database processing by all the grantees. Access to the cloud and supercomputing services (Amazon) is provided by core funding to EcoHealth Alliance.

Institute of Pathogen Biology (Ren, Guo)

The Institute of Pathogen Biology laboratories have equipment required for general microbiological, molecular, and biochemical work including microcentrifuges, agarose and polyacrylamide electrophoresis equipment, spectrophotometer, rocking and shaking platforms, bead-beater cell disruptor, and incubators (shaking and static). Major equipment relevant to this proposal which are available include:

BSL2 Facility. The institute has three laboratories of [redacted] (b) (4) equipped as BSL2 space for the virology, immunology and clinical samples pretreatment. Equipment includes Illumina Hiseq 2500, Miseq and BGI 500, gel electrophoresis equipment, power supplies, thermal cyclers, a programmable heat block, heat blocks, water baths, CO_2 incubators (2), several -70°C freezers, one -140°C freezer, refrigerators, DNA documentation system, DNA sequencing and computer assisted sequence analysis programs, several microfuges, Nikon and Zeiss microscopes with photographic and fluorescent capabilities, several class 2 environmental hoods, refrigerated water baths, real time thermocyclers, and spectrophotometer. The laboratory has an ELISA plate reader, an illuminometer, ELISA plate washer, spectrophotometers, and other equipment that is routinely used in characterizing antibody-protein interactions.

BSL 3 Facility. The institute shares an additional [redacted] (b) (4) of BSL3 facilities equipped with sterile hoods (BSCIIA), CO_2 incubators, -70°C freezer, an inverted Nikon fluorescent microscope with an assortment of filters, magnifications and digital camera, and related equipment necessary for virus cultivation and molecular genetic research.

Wuhan Institute of Virology (Shi, Zhou, Hu)

Institute of Virology's Emerging Virus Laboratory has equipment required for general microbiological, molecular, and biochemical work including microcentrifuges, agarose and polyacrylamide electrophoresis equipment, spectrophotometer, rocking and shaking platforms, bead-beater cell disruptor, and incubators (shaking and static). Major equipment relevant to this proposal which are available include: -80°C freezers, PCR machines, gel electrophoresis and imaging system, biosafety cabinets, super-clean benches, and cell culture rooms.

A Core Facility Center was established at Wuhan Institute of Virology to provide technological services to faculty, students, and visiting researchers. The equipment installed in the Core Facility Center include: transmission electron microscope, ultracentrifugation machines, small animal *in vivo* imaging systems, confocal laser scanning microscopes, flow cytometry, a real-time qPCR system, and a high-throughput sequencing and analyzing system.

In addition, the Wuhan Institute of Virology owns a complete biosafety research platform, which consists of the first national BSL-4 laboratory in China, and a cluster of BSL-3 and BSL-2 labs. These labs contain gel electrophoresis equipment, power supplies, thermal cyclers, programmable heat blocks, heat blocks, water

baths, CO_2 incubators, -70°C freezers, -140°C freezers, refrigerators, DNA documentation system, DNA sequencing and computer assisted sequence analysis programs, microfuges, Nikon and Zeiss microscopes with photographic and fluorescent capabilities, several class 2 environmental hoods, refrigerated water baths, real time thermocyclers, and spectrophotometers. The laboratory also has an ELISA plate reader, an illuminometer, ELISA plate washer, spectrophotometers, and other equipment that is routinely used in characterizing antibody-protein interactions.

University of North Carolina at Chapel Hill Baric Laboratory (Baric, Sims)
The three laboratories of the Baric Lab in the Department of Epidemiology have equipment required for general microbiological, molecular, and biochemical work including microcentrifuges, agarose and polyacrylamide electrophoresis equipment, spectrophotometer, rocking and shaking platforms, bead-beater cell disruptor, and incubators (shaking and static). Major equipment relevant to this proposal which are available include: gel electrophoresis equipment, power supplies, thermal cyclers, a programmable heat block, heat blocks, water baths, CO_2 incubators (2), several -70°C freezers, one -140°C freezer, refrigerators, DNA documentation system, DNA sequencing and computer assisted sequence analysis programs, several microfuges, two Nikon microscopes with photographic and fluorescent capabilities, several class 2 environmental hoods, refrigerated water baths, 10+ IBM and Apple Pentium II/III computers with accompanying software, three thermocyclers, a fume hood, Nuclisens reader, hybridization oven, real time thermocyclers, three fluorescent inverted scopes with computer software (Olympus IX51), and a spectrophotometer. A Roche Light Cycler 480II is available for real time measurements. The laboratory has an ELISA plate reader, an illuminometer, 200 cages for animal maintenance and breeding in Seal-Safe housing, Bio Rad low pressure chromatography system, ELISA plate washer, spectrophotometers, and other equipment that is routinely used in characterizing antibody-protein interactions.

BSL3 Facility features include: shower in/shower out facility; dual anteroom access; Hepa filtered exhaust; redundant exhaust fans; card key access; an alarm system to Public Health/Campus Police; laboratory controlled combination lock; and Techniplast Sealsafe™ Hepa filtered animal housing for 300+ rodents. PAPR and tyvek suits are worn at all times in the BSL3 facility. The BL3 facilities are in an adjacent and attached building [redacted] (b) (4) or in [redacted] (b) (4), the latter space is directly adjacent to Dr. Baric's BSL2 laboratory resources. Each facility is equipped with sterile hoods (BSCIIA), four CO_2 incubators, gel electrophoresis equipment, thermal cyclers and power supplies, and related equipment necessary for virus cultivation and molecular genetic research. The facilities each house a -70°C freezer, an inverted Nikon fluorescent microscope with an assortment of filters, magnifications and digital camera, an ELISA plate reader and illuminometer. Both facilities contain rodent-sized Seal-Safe systems (~192 cages) for maintaining animals in a Hepa-filtered Air in/out environment, exhausted into the BSL3 Hepa-filtered exhaust system. An 8 chamber Buxco plethysmography system that allows for repetitive, noninvasive measures of the number of breaths, tidal volume, airway responsiveness, enhanced pause, and respiratory gases from live control and infected mice in a contained system is housed in the main BSL3 laboratory in [redacted] (b) (4)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

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Project Role*: PD/PI Other Project Role Category:

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Attach Biographical Sketch*: File Name: DASZAK_Peter_Biosketch_Final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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Attach Biographical Sketch*: File Name: SHI_Zhengli_Biosketch_Final.pdf

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PROFILE - Senior/Key Person

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Degree Type: PHD Degree Year: 2008

Attach Biographical Sketch*: File Name: OLIVAL_Kevin_Biosketch_Final.pdf

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PROFILE - Senior/Key Person

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Degree Type: PHD Degree Year: 1977

Attach Biographical Sketch*: File Name: BARIC_Ralph_Biosketch_Final.pdf

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Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD Degree Year: 2015

Attach Biographical Sketch*: File Name: ROSS_Noam_Biosketch_Final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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Project Role*: Other (Specify) Other Project Role Category: Research Scientist

Degree Type: PHD Degree Year: 2012

Attach Biographical Sketch*: File Name: LATINNE_Alice_Biosketch_Final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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Project Role*: Other (Specify) Other Project Role Category: Research Scientist

Degree Type: MPH Degree Year: 2015

Attach Biographical Sketch*: File Name: LI_Hongying_Biosketch_Final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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Degree Type: PHD Degree Year: 2010

Attach Biographical Sketch*: File Name: FRANCESCO_Leilani_Biosketch_Final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD Degree Year: 2001

Attach Biographical Sketch*: File Name: SIMS_Biosketch_Final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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Project Role*: Other (Specify) Other Project Role Category: Research Scientist

Degree Type: MPH Degree Year: 2013

Attach Biographical Sketch*: File Name: HAGAN_Emily_Biosketch_Final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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State*:
Province:
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E-Mail*: (b) (6)

Credential, e.g., agency login: (b) (6)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD Degree Year: 2012

Attach Biographical Sketch*: File Name: ZHU_GuangJian_Biosketch_Final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Linfa Middle Name Last Name*: Wang Suffix:

Position/Title*: Professor & Director
Organization Name*: Duke-NUS Medical School
Department:
Division:
Street1*: 8 College Road
Street2:
City*: Singapore
County:
State*:
Province:
Country*: SGP: SINGAPORE
Zip / Postal Code*: 169857

Phone Number*: [redacted] (b) (6) Fax Number:

E-Mail*: [redacted] (b) (6)

Credential, e.g., agency login: [redacted] (b) (6)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD Degree Year: 1986

Attach Biographical Sketch*: File Name: WANG_Linfa_Final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Lili Middle Name Last Name*: Ren Suffix:

Position/Title*: Research Scientist
Organization Name*: Institute of Pathogen Biology
Department:
Division:
Street1*: No. 9 Dong Dan San Tiao
Street2: Dongcheng District
City*: Beijing
County:
State*:
Province:
Country*: CHN: CHINA
Zip / Postal Code*: 100730

Phone Number*: [redacted] (b) (6) Fax Number:

E-Mail*: [redacted] (b) (6)

Credential, e.g., agency login: [redacted] (b) (6)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD Degree Year: 2005

Attach Biographical Sketch*: File Name: REN_Lili_Biosketch_Final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Li Middle Name Last Name*: Guo Suffix:

Position/Title*: Professor
Organization Name*: Institute of Pathogen Biology
Department:
Division:
Street1*: No. 9 Dong Dan San Tiao
Street2: Dongcheng District
City*: Beijing
County:
State*:
Province:
Country*: CHN: CHINA
Zip / Postal Code*: 100730

Phone Number*: [redacted] (b) (6) Fax Number:

E-Mail*: [redacted] (b) (6)

Credential, e.g., agency login: [redacted] (b) (6)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: MD Degree Year: 2006

Attach Biographical Sketch*: File Name: GUO_Li_Biosketch_Final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Peng Middle Name Last Name*: Zhou Suffix:

Position/Title*: Principal Investigator
Organization Name*: Wuhan Institute of Virology
Department:
Division:
Street1*: Xiao Hong Shan, No. 44
Street2:
City*: Wuhan
County:
State*:
Province:
Country*: CHN: CHINA
Zip / Postal Code*: 430071

Phone Number*: [redacted] (b) (6) Fax Number:

E-Mail*: [redacted] (b) (6)

Credential, e.g., agency login: [redacted] (b) (6)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD Degree Year: 2011

Attach Biographical Sketch*: File Name: ZHOU_Peng_Biosketch_Final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Ben Middle Name Last Name*: Hu Suffix:

Position/Title*: Research Scientist
Organization Name*: Wuhan Institute of Virology
Department:
Division:
Street1*: Xiao Hong Shan, No. 44
Street2:
City*: Wuhan
County:
State*:
Province:
Country*: CHN: CHINA
Zip / Postal Code*: 430071

Phone Number*: [redacted] (b) (6) Fax Number:

E-Mail*: [redacted] (b) (6)

Credential, e.g., agency login: [redacted] (b) (6)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD Degree Year: 2015

Attach Biographical Sketch*: File Name: HU_Ben_Biosketch_final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Aleksei Middle Name Last Name*: Chmura Suffix:

Position/Title*: Research Scientist
Organization Name*: EcoHealth Alliance
Department:
Division:
Street1*: 460 West 34th Street
Street2: Suite 1701
City*: New York
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 100012317

Phone Number*: [redacted] (b) (6) Fax Number: +12123804465

E-Mail*: [redacted] (b) (6)

Credential, e.g., agency login: [redacted] (b) (6)

Project Role*: Other (Specify) Other Project Role Category: Research Scientist

Degree Type: PHD Degree Year: 2018

Attach Biographical Sketch*: File Name: CHMURA_Aleksei_Biosketch_Final.pdf

Attach Current & Pending Support: File Name:

BIOGRAPHICAL SKETCH

DO NOT EXCEED FIVE PAGES.

NAME: Peter Daszak

eRA COMMONS USER NAME (credential, e.g., agency login): (b) (6)

POSITION TITLE: President & Chief Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Bangor University, UK	B.S (hons)	07/1986	Zoology
University of East London, UK	Ph.D.	03/1993	Infectious Diseases

A. Personal Statement

I have the broad expertise in emerging viral zoonoses, and scientific management experience to support this proposed work that involves an international interdisciplinary team working on field collection of wildlife and human samples, human behavioral risk surveys, modeling and analytics, and viral characterization *in vitro* and *in vivo*. I am President and Chief Scientist of EcoHealth Alliance, a US-based 501 (c) 3 institution that conducts research on emerging zoonoses and global health capacity building. My 20+ years of NIH-funded research focuses on understanding the links among disease emergence in wildlife, livestock and people, particularly viral zoonoses. This includes identifying the bat origin of SARS-CoV and SADS-CoV, analyzing the ecology of West Nile, Nipah and Hendra virus emergence, publishing the first unbiased analysis of global emerging disease hotspots, and developing the scientific rationale for the Global Virome Project (GVP). Over the past 18 years I have been the PI on 4 multidisciplinary R01s that use modeling, epidemiology, laboratory and field science to test hypotheses on the emergence of wildlife-origin viral zoonoses, including SARS-CoV, Nipah and Hendra virus, Avian influenza and novel viruses from bats. I have successfully managed teams of virologists, field biologists, mathematical modelers, veterinarians, epidemiologists, laboratorians and anthropologists. Much of the groundwork for the current proposal has developed from my previous collaborative research with each member of the team assembled in the current R01 renewal proposal.

1. Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, Wang H, Crameri G, Hu Z, Zhang H, Zhang J, McEachern J, Field H, **Daszak P**, Eaton BT, Zhang S & Wang L-F (2005). Bats are natural reservoirs of SARS-like coronaviruses. **Science** 310: 676-679.
2. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, and **Daszak P*** (2008). Global trends in emerging infectious diseases. **Nature** 451:990-993
3. Olival KJ*, Hosseini PR, Zambrana-Torrelio C, Ross N, Bogich TL, **Daszak P*** (2017). Host and viral traits predict zoonotic spillover from mammals. **Nature** 546, 646–650.
4. Carroll D, **Daszak P***, Wolfe ND, Gao GF, Morel C, Morzaria S, Pablos-Méndez A, Tomori O, Mazet JAK (2018). The global virome project. **Science** 359: 872-874.

B. Positions and Honors

Positions and Employment

1993 -98	Senior Faculty Research Scientist, Kingston University UK
1998	Guest Researcher, Centers for Disease Control and Prevention (CDC)
1999 -01	Faculty Research Scientist, University of Georgia
2001 -	Sr. Adjunct Faculty, Columbia University
2001 - 09	Executive Director, Consortium for Conservation Medicine, EcoHealth Alliance, New York
2009 -	President & Chief Scientist, EcoHealth Alliance New York

Other Experience and Professional Membership

2003 - 7	NIH: ad hoc member, ZRG1 IDM-G 90 (2003-5) ZRG1 IRAP-Q (2005-7)
2004 -	Editorial Board, *Conserv. Biol.*
2005	NIAID: Steering Committee, workshop on virus-host shifts & emergence of new pathogens
2010 -	Editor-in-Chief, *EcoHealth*; Member of IOM Forum on Microbial Threats; External Advisory Board, DHS and Kansas State Univ. Ctr. of Excellence for Emerg. & Zoonotic Animal Diseases (CEEZAD)
2011	Steering Committeee, NIAID Workshop on Arboviruses
2014 -	Member NRC Advisory Committee to advise the US Global Change Research Program (USGCRP)
2015 -	Member of Supervisory Board, One Health Platform; Editorial Board *One Health*
2016 -	Member, WHO Expert group on Public Health Emergency Disease Prioritization
2016 -	Member, Core Steering Committee & Co-Chair, Science & Technol WG, Global Virome Project
2017	External Review Committee, CSIRO Health & Biosecurity Business Unit
2017 -	Chair, Forum on Microbial Threats, National Academies of Science, Engineering & Medicine

Honors

1999	Meritorious service award, CDC
2000	CSIRO silver medal for collaborative research
2002	Honored by the naming of a new species of centipede, *Cryptops daszaki* (*J Nat Hist* 36: 76-106)
2003	6th Annual Lecturer, Medicine & Humanities, Texas A&M
2007	Finalist, Director's Pioneer Award
2008	Presidential Lecturer, University of Montana
2012	Elected member of the Cosmos Club, Washington DC
2013	Honored by the naming of a new parasite species, *Isospora daszaki* (*Parasit. Res.* 111:1463-1466)
2013	Hsu-Li Distinguished Lectureship in International Epidemiology, Univ. Iowa
2015	Robert Leader Endowed Lecture in Food Safety, Michigan State Univ.
2018 -	Member, National Institute of Medicine (NAM), USA.

C. Contribution to Science

1. Research on the bat origins of emerging viruses. A range high impact emerging viruses appear to have bat reservoirs (e.g. SARS-CoV, EBOV, NiV, HeV, MERS-CoV, SADS-CoV). As PI on four prior R01s, my work has helped demonstrate the bat-origin for some of these (SARS-CoV, SADS-CoV), analyze the drivers of emergence and risk factors for spillover. Collaborating with virologists in China, we have isolated and characterized SARS-like CoVs from bats that use the same human host cell receptor (ACE-2) as SARS-CoV. This work provides critical reagents and resources that have helped advance understanding of virus-host binding and may contribute to vaccine development. My other work identified factors underlying the emergence of NiV from *Pteropus* bats in Malaysia and Bangladesh; that MERS-CoV likely originated in bats; that SADS-CoV originates in bats; and that bats harbor a significantly higher proportion of zoonoses than all other mammalian groups after correcting for reporting biases.

a. Pulliam JRC, Epstein JH, Dushoff J, Rahman SA, Bunning M, HERG, Jamaluddin AA, Hyatt AD, Field HE, Dobson AP & **Daszak P*** and the Henipavirus Ecology Research Group (HERG). (2012). Agricultural intensification, priming for persistence, and the emergence of Nipah virus: a lethal bat-borne zoonosis. **J Roy Soc Interface** 9:89-101

b. Ge X-Y, Li J-L, Yang X-L, Chmura AA, Zhu G, Epstein JH, Mazet JK, Hu B, Zhang W, Peng C, Zhang Y-J, Luo C-M, Tan B, Wang N, Zhu Y, Crameri G, Zhang S-Y, Wang L-F, **Daszak P***, Shi Z-L* (2013). Isolation and characterization of a bat SARS-like Coronavirus that uses the ACE2 receptor. **Nature** 503: 535-538.

c. Memish ZA, Mishra N, Olival KJ, Fagbo SF, Kapoor V, Epstein JH, Al Hakeem R, Durosinloun A, Al Asmari M, Islam A, Kapoor A, Briese T, **Daszak P**, Al Rabeeah A, Lipkin WI. (2013). Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. **EID** 19(11): 1819-1823.

d. Zhou P, Fan H, Lan T, Yang X-L, Shi W-F, Zhang W, Zhu Y, Zhang Y-W, Xie Q-M, Mani S, Zheng X-S, Li B, Li J-M, Guo H, Pei G-Q, An X-P, Chen J-W, Zhou L, Mai K-J, Wu Z-X, Li D, Anderson DE, Zhang L-B, Li S-Y, Mi Z-Q, He T-T, Cong F, Fuo P-J, Huang R, Luo Y, Liu X-L, Chen J, Huang Y, Sun Q, Zhang X-L-L, Wang Y-Y, Xing S-Z, Chen Y-S, Sun Y, Li J, **Daszak P***, Wang L-F*, Shi Z-L*, Tong Y-G*, Ma J-Y* (2018). Fatal Swine Acute Diarrhea Syndrome caused by an HKU2-related Coronavirus of Bat Origin. **Nature** 556: 255-258.

2. **Analyzing the process of disease emergence.** Emerging infectious diseases are a significant threat to global health. However, their emergence is sporadic, complex, and seemingly unpredictable. In the early 2000s I started to use analytical approaches to see if there are patterns in disease emergence, and if these are predictable. By collating a database of all known prior EID events, identifying their point origins, and correcting for reporting biases, I published the first ever predictive 'hotspots' maps of where disease emergence is most likely. Under various grants that I have led, or been a co-investigator on, I have published spatial analyses of the drivers of disease spread, and strategies to predict pandemic emergence.

   a. Kilpatrick AM, Chmura AA, Gibbons DW, Fleischer RC, Marra PP & **Daszak P** (2006). Predicting the global spread of H5N1 avian influenza. **PNAS** 103: 19368-19373.

   b. Morse SS, Mazet JAK, Woolhouse M, Parrish CR, Carroll D, Karesh WB, Zambrana-Torrelio C, Lipkin WI, **Daszak P*** (2012). Prediction and prevention of the next pandemic zoonosis. **Lancet** 380:1956-1965.

   c. **Daszak P***, Zambrana-Torellio C, Bogich TL, Fernandez M, Epstein JH, Murray KA, Hamilton H (2013). Interdisciplinary approaches to understanding disease emergence: The past, present and future drivers of Nipah virus emergence. **PNAS** 110: 3681-3688

   d. Allen T, Murray KA, Zambrana-Torrelio C, Morse SS, Rondinini C, Di Marco M, Breit N, Olival KJ, **Daszak P*** (2017). Global hotspots and correlates of emerging zoonotic diseases. **Nature Comm** 8: 1124

3. **Studies of wildlife disease ecology to understand emerging zoonoses.** The majority of EIDs are zoonotic, with the majority of these originating in wildlife. In the 1990s, new collaborations among ecologists and medical researchers began to show that understanding disease dynamics in wildlife can allow better forecasting of disease risk in people. I reviewed this field in a paper in *Science* in 2000 and in a more recent paper in *Nature* on the links among biodiversity and health. During the last two decades, I have led collaborative research programs on how the ecology of specific wildlife-origin zoonoses can help explain patterns of risk to people. This includes my work in 4 R01s and as EHA institutional lead for USAID-EPT-PREDICT, and Chief of Party for USAID-IDEEAL. This work has led to strategies to estimate the diversity of yet-to-be discovered viruses, and a program to identify them (the Global Virome Project).

a. **Daszak P***, Cunningham AA, Hyatt AD (2000). Emerging infectious diseases of wildlife - threats to biodiversity and human health. **Science** 287: 443-449

b. Keesing F, Belden LK, **Daszak P**, Dobson A, Harvell CD, Holt RD, Hudson P, Jolles A, Jones KE, Mitchell CE, Myers SS, Bogich T & Ostfeld RS. (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. **Nature** 468:647-652.

c. Anthony SJ, Epstein JH, Murray KA, Navarrete-Macias I, Zambrana-Torrelio CM, Solovyov A, Ojeda-Flores R, Arrigo NC, Islam A, Ali Khan S, Hosseini P, Bogich TL, Olival KJ, Sanchez-Leon MD, Karesh W, Goldstein T, Luby SP, Morse SS, Mazet JAK, **Daszak P**, Lipkin WI. (2013). A strategy to estimate unknown viral diversity in mammals. **MBio** 4(5): e00598-13.

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

USAID Emerging Pandemic Threats | Mazet (PI) | 10/01/14 – 09/30/19
PREDICT-2
The goal of this work is to conduct surveillance for novel pathogens in wildlife, livestock and people; characterize human risk behavior; analyze EID risk; and design interventions in >20 countries
Role: PI on Subcontract

1R01 AI110964 | Daszak (PI) | 06/01/14 – 05/31/19
Understanding the Risk of Bat Coronavirus Emergence
The goal of this work is to conduct ecological and virological studies on bats in China that harbor SARS-like coronaviruses, and conduct behavioral risk surveys and testing in people, with a goal of identifying risk factors for further spillover of SARS-like CoVs, and help identify the likely drivers of the SARS-CoV outbreak in 2003.
Role: PI

USAID 1414374 (RDMA, Thailand) | Daszak (CoP) | 10/01/13 - 03/30/19
Infectious Disease Emergence and Economics of Altered Landscapes (IDEEAL)
The goal of this cooperative agreement is to analyze how land use change affects disease risk in SE Asia, and how economic costs of disease can be used to develop novel intervention policies.
Role: Chief of Party

Completed Research Support

NSF DEB 1414374 | Perrings (PI) | 10/15/14 - 04/14/18
US-UK Collab: Risks of Animal and Plant Infectious Diseases through Trade (RAPID Trade)
The goal of this NSF-NIH-USDA EEID award, joint with a UK BBSRC grant is to analyze and model how policy changes to trade affect emerging disease risk globally
Role: Co-Investigator

HDTRA1 | Allen (PI) | 04/15/15 - 04/14/17
Global Rapid Identification of undiagnosed EID Events
The goal of this project was to design software that can be used in the DoD biosurveillance ecosystem (BSVE) to rapidly diagnose novel EID events.
Role: Co-Investigator

1R01GM100471 (NIGMS) | Perrings (PI) | 09/15/11-06/30/15
MASpread: Modeling Anthropogenic Effects in the Spread of Infectious Disease
The goal of this project was to develop novel approaches to modeling and analyzing disease spread and the social decisions involved in control
Role: Co-Investigator

NSF | Daszak (PI) | 07/01/10-06/30/15

EcoHealthNet - a Research Coordination Network
Funding for student exchange and workshops to fuse veterinary science, ecology and human medical sciences
Role: PI

USAID Emerging Pandemic Threats Mazet (PI) 10/01/09 – 09/30/14
PREDICT-1
The goal of this work was to conduct surveillance for novel pathogens in wildlife, livestock and people in developing countries
Role: PI on Subcontract

2 R01TW005869 Daszak (PI) 09/01/08 – 08/31/13
The Ecology, Emergence and Pandemic Potential of Nipah virus in Bangladesh
This project involved mathematical modeling and fieldwork on the dynamics of Nipah virus in Bangladesh
Role: PI

NSF DEB-1257513 Daszak (PI) 08/15/12-07/31/13
US-China Ecology and Evolution of Infectious Diseases Collaborative Workshop; Kunming, China
The goal of this work was to organize a workshop among NIH, NSF, leading US and Chinese scientists to discuss potential for a jointly funded NIH-NSF-China funding mechanism
Role: PI

1 R01AI079231 (NIAID) Daszak (PI) 09/18/08 – 08/31/13
Risk of viral emergence from bats.
The goal was to model hotspots for bat viral diversity, identify & characterize new bat viruses & understand their pathology
Role: PI

NSF BCS 0826779 Daszak (PI) 10/01/08 – 03/31/12
AOC - HSD – Collaborative Research: Human-related factors affecting emerging infectious diseases
The goal of this work was to analyze how socio-economic and environmental drivers predict risk of EIDs
Role: PI on lead proposal

R01TW005869 - supplemental Daszak (PI) 09/01/08 – 08/31/11
Supplemental funding: Predicting the risk of global H5N1 spread
This project involved mathematical modeling and fieldwork in Bangladesh and China to understand risk of H5N1 spread.
Role: PI

NSF EF-062239 Kilpatrick (PI) 09/01/06 - 08/30/11
Predicting spatial variation in West Nile virus transmission
The goal was to study interaction among WNV vector, reservoir host populations across an urban-to-rural gradient.
Role: Co-PI

R01 TW05869 (Fogarty Intl. Ctr.) Daszak (PI) 08/01/02 - 05/31/07
Anthropogenic change & emerging zoonotic paramyxoviruses
The goal was to identify the cause of emergence of Nipah and Hendra viruses in Malaysia and Australia.
Role: PI

NSF HSD 0525216 Daszak (PI) 10/15/05 - 10/14/06
Collaborative Research: Socio-Economic and Environmental Drivers of Emerging Diseases
The goal of this work was to analyze patterns of disease emergence globally leading to development of a global hotspots map of disease emergence.
Role: PI

BIOGRAPHICAL SKETCH

NAME Zhengli Shi	POSITION TITLE Co-Investigator
eRA COMMONS USER NAME (credential, e.g., agency login) (b) (6)	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
Department of Biology, Wuhan University, China	BS	1987	Genetics
Wuhan Inst. Virol., Chinese Acad. Sci., China	MS	1990	Virology
University Montpellier II, Montpellier, France	Ph.D.	2000	Virology

A. Personal Statement

I have been working on the discovery and characterization of novel viruses from bats and other wildlife since 2004. This included the discovery that Chinese horseshoe bats are the natural reservoir of SARSr-CoVs and the likely origin of SARS-CoV. My group then isolated SARSr-CoVs from bats sharing high homology with human SARS-CoV and demonstrated their interspecies transmission risk, largely confirming bats as the source of SARs. My lab has carried out systematic studies on the epidemiology, genetic evolution, interspecies infection mechanism and pathogenesis of a series of bat-borne emerging viruses including SARSr-CoV, MERS-CoV, EBOV and others. This work has involved collaboration on all other scientists on this R01 renewal proposal, in particular Drs. Daszak and Linfa Wang, who I have collaborated with since 2003, publishing 2 papers in *Nature* and one in *Science* together on our bat-virus work, as well as dozens of others. Recently, this collaborative team discovered that an outbreak of fatal Swine Acute Diarrhea Syndrome in southern China that killed more than 24,000 piglets was caused by spillover of bat HKU2-related coronaviruses. In this proposed work, my group will be responsible for CoV testing in bat samples, serological testing in human samples, and virus characterization work such as cell entry analysis and receptor identification.

B. Positions and Honors.

Positions and Employment

1990 - 93	Research assistant, Wuhan Institute of Virology, Chinese Academy of Sciences, China
1993 - 95	Research scientist, Wuhan Institute of Virology, Chinese Academy of Sciences, China
2000 -	Senior Scientist, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China

Other Experience and Professional Memberships

2011 -	Director, Center for Emerging Infectious Diseases, Wuhan Inst. Virology, Chinese Acad. Sci.
2013 -	Director, BSL-3 laboratory at Wuhan Institute of Virology, Chinese Acad. Sci.
2014 -	Director, Committee of Biosafety, Wuhan Institute of Virology, Chinese Acad. Sci.
2014 -	Director, CAS Key Laboratory of Special Pathogens and Biosafety
2015 -	Vice Director, BSL-4 laboratory, Wuhan Institute of Virology, Chinese Acad. Sci.
2016 - 18	Associate Editor of *Virology Journal*
2017 - 19	Editorial Board of *Virology*
2017-2019	Editor in Chief, *Virologica Sinica*

Honors

2003 Natural Science Award (the Second Prize) of Hubei Province, China.
2004 Outstanding supervisor of graduate student of Hubei Province, China.
2006 Outstanding scientist of the Chinese Academy of Sciences.
2006 Outstanding Research Article on Natural Science (the First Prize), Hubei Province, China
2014 Young and Middle-aged Scholar with Distinguished Contribution in Hubei Province, China
2014 Outstanding Research Article on Natural Science (the Grand Prize), Hubei Province, China
2016 Palm Knight Medal for Education, Government of the Republic of France
2017 Natural Science Award (the First Prize) of Hubei Province, China.

C. Selected peer-reviewed publications most relevant to the current application

* = Co-corresponding or first author

Li W*, Shi Z*, Yu M, Ren W, Smith C, Epstein HJ, Wang H, Crameri G, Hu Z, Zhang H, Zhang J, Mceachern J, Field H, Daszak P, Eaton TB, Zhang S, Wang LF (2005). Bats are natural reservoirs of SARS-like coronaviruses. **Science**, 310: 676-679.

Ren W, Qu X, Li W, Han Z, Yu M, Zhang S, Wang LF, Deng H, Shi Z (2008) Difference in receptor usage between SARS coronavirus and SARS-like coronavirus of bat origin. **Journal of Virology** 82(4): 1899–1907.

Yuan J, Hon CC, Li Y, Wang D, Xu G, Zhang H, Zhou P, Poon LM, Lam TT, Leung FC. Shi Z (2010). Intra-species Diversity of SARS-Like Coronaviruses (CoVs) in *Rhinolophus sinicus* and Its Implications on the Origin of SARS-CoVs in human. **Journal of General Virology**, 91(4):1058-1062.

Ge XY, Li JL, Yang X-L, Chmura AA, Zhu G, Epstein JH, Mazet JK, Hu B, Zhang W, Peng C, Zhang YJ, Luo CM, Tan B, Wang N, Zhu Y, Crameri G, Zhang SY, Wang LF, Daszak P*, Shi Z* (2013). Isolation and characterization of a bat SARS-like Coronavirus that uses the ACE2 receptor. **Nature** 503: 535-538.

Menachery VD, Yount BL, Debbink K, Agnihothram S, Gralinski LE, Plante JA, Graham RL, Scobey T, Ge XY, Donaldson EF, Randell SH, Lanzavecchia A, Marasco WA, Shi Z*, Baric RS* (2015). A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. **Nature Medicine**, 21:1508-1513.

Yang XL, Hu B, Wang B, Wang MN, Zhang Q, Zhang W, Wu LJ, Ge XY, Zhang YZ, Daszak P, Wang LF*, Shi Z* (2016). Isolation and Characterization of a Novel Bat Coronavirus Closely Related to the Direct Progenitor of Severe Acute Respiratory Syndrome Coronavirus. **Journal of Virology**, 90: 3253-3256.

Zeng L, Ge X, Peng C, Yang X, Tan B, Gao Y, Chen J, Chmura AA, Daszak P*, Shi Z* (2016) Bat Severe Acute Respiratory Syndrome-Like Coronavirus WIV1 Encodes an Extra Accessory Protein, ORFX, Involved in Modulation of the Host Immune Response. **Journal of Virology**, 90(14): 6573–6582.

Hu B, Zeng LP, Yang XL, Ge XY, Zhang W, Li B, Xie JZ, Shen XR, Zhang YZ, Wang N, Luo DS, Zheng XS, Wang MN, Daszak P, Wang LF, Cui J*, Shi Z* (2017). Discovery of A Rich Gene Pool of Bat SARS-related Coronaviruses Provides New Insights into the Origin of SARS Coronavirus. **PLOS Pathogens**, 13(11): e1006698.

Zhou P, Fan H, Lan T, Yang XL, Shi WF, Zhang W, Zhu Y, Zhang YW, Xie QM, Mani S, Zheng XS, Li B, Li JM, Guo H, Pei GQ, An XP, Chen JW, Zhou L, Mai KJ, Wu ZX, Li D, Anderson D, Zhang LB, Li SY, Mi ZQ, He TT, Cong F, Guo PJ, Huang R, Luo Y, Liu XL, Chen J, Huang Y, Sun Q, Zhang XLL, Wang YY, Xing SZ, Chen YS, Sun Y, Li J, Daszak P, Wang LF, Shi Z, Tong YG, Ma JY (2018) Fatal swine acute diarrhea syndrome caused by an HKU-2 related coronavirus of bat origin. **Nature**, 556: 255-258.

Luo CM, Wang N, Yang XL, Liu HZ, Zhang W, Li B, Hu B, Peng C, Geng QB, Zhu G, Li F*, Shi Z* (2018). Discovery of Novel Bat Coronaviruses in South China That Use the Same Receptor as Middle East Respiratory Syndrome Coronavirus. **Journal of Virology**, 92 (13): e00116-18.

Additional recent publications of importance to the field (in chronological order)

Ge X, Li Y, Yang X, Zhang H, Zhou P, Zhang Y, Shi Z (2012). Metagenomic analysis of viruses from bat fecal samples reveals many novel viruses in insectivorous bats in china. **Journal of Virology**, 86, 4620-4630.

Yuan J, Zhang Y, Li J, Zhang Y, Wang LF*, Shi Z* (2012). Serological evidence of ebolavirus infection in bats, China. **Virology Journal**, 9: 236.

Yang XL, Zhang YZ, Jiang RD, Guo H, Zhang W, Li B, Wang N, Wang L, Waruhiu C, Zhou JH, Li SY, Daszak P, Wang LF*, Shi Z* (2017). Genetically Diverse Filoviruses in *Rousettus* and *Eonycteris* spp. Bats, China, 2009 and 2015. **Emerging Infectious Diseases**, 23(3):482-486.

Zeng LP, Ge XY, Peng C, Tai WB, Jiang SB, Du LY*, Shi Z* (2017). Cross-neutralization of SARS coronavirus-specific antibodies against bat SARS-like coronaviruses. **Science China Life Sciences**, 60(12):1399-1402.

Wang N, Li SY, Yang XL, Huang, HM, Zhang YJ, Guo H, Luo CM, Miller M, Zhu G, Chmura AA, Hagan E, Zhou JH, Zhang YZ, Wang LF, Daszak P*, Shi Z* (2018). Serological Evidence of Bat SARS-Related Coronavirus Infection in Humans, China. **Virologica Sinica**, 33(1):104-107.

D. Research Support
Ongoing Research Support

[redacted] (b) (4)

Geographical distribution and genetic variation of pathogens in Africa
Role: PI

31770175 National Natural Science Foundation of China 01/01/2018-12/31/2021
Evolution mechanism of the adation of bat SARS-related coronaviruses to host receptor molecules and the risk of interspecies infection
Role: PI

[redacted] (b) (4)

Genetic evolution and transmission mechanism of important bat-borne viruses
Role: PI

R01 AI110964 Daszak (PI) 06/01/14-05/31/19
Understanding Risk of Bat Coronaviruses
The goal of this study is to analyze the risk of coronavirus spillover from bats to humans in Southern China
Role: Co-Investigator

Emerging Pandemic Threat Program, USAID Mazet (PI) 10/01/14-09/30/19
PREDICT 2
The goal of this project is to create and implement a global virus surveillance system in animals and humans and analyze spillover risk.
Role: China Country Coordinator

Completed Research Support

[redacted] (b) (4)

Metagenomic analysis of bat intestinal viruses
Role: PI

[redacted] (b) (4)

Mechanism of interspecies transmission of zoonotic viruses

Role: Co-PI

[redacted] (b) (4)

Genetic diversity, identification and pathogenesis of bat viruses [redacted] (b) (4)

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Kevin J. Olival	Co-Investigator
eRA COMMONS USER NAME (b) (6) [redacted]	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
Colorado State University, Fort Collins, CO	BS	05/1997	Biology
Columbia University, New York, NY	MA	10/2003	Conservation Biology
Columbia University, New York, NY	PhD	05/2008	Ecology & Evolution
American Museum of Natural History, New York	Post Doc	08/2009	Molecular Parasitology
NIH Fogarty US Global Health Fellow, New York	Post Doc	08/2011	EIDs

A. Personal Statement

The goal of this proposal is to understand the current and future threat of bat-borne coronavirus spillover in Southern China, by identifying which viruses, host species, and human behaviors are associated with the highest risk of CoV exposure. Specifically, we will use a combination of targeted bat sampling, human behavioral risk analyses, mathematical modeling, and phylogenetic and molecular methods to test several hypotheses related to zoonotic spillover risk of β-CoVs, with specific attention paid towards SARSr-CoVs. My research experience over the last 16 years on bat-borne disease evolution, ecology, dynamics, population genetics, and viral discovery is strongly complementary to these aims. Our current proposal builds upon the findings of an ongoing NIAID R01 grant (ending 5/31/19), for which I was a co-investigator. Prior to this I coordinated research efforts under a NIAID award (2011-2016), investigating the risk of viral emergence from bats. This included sample collection and testing of thousands of bats from 8 countries globally. As an NIH Fogarty Global Health Post-Doc Fellow, I gained invaluable experience working internationally with a project focused on the ecology and evolution of Nipah virus in Bangladesh. My work over the last decade includes leading field investigations and bat viral surveillance in a wide range of countries, including: Bangladesh, Cambodia, India, Indonesia, Malaysia, Thailand, Philippines, Saudi Arabia, Georgia, Jordan, and Turkey. Discoveries include the first viral isolation of Nipah virus from the large flying fox in Malaysia; evidence of MERS-CoV in bats in Saudi Arabia; and the first serological evidence of Ebola Zaire virus in bats in Asia. I currently serve as the Modeling & Analytics coordinator under the USAID PREDICT-2 project, working with a team of analyst to develop new approaches to predict and prevent zoonoses. As part of this effort, I developed a new approach that combines phylogenetic, ecological, and life-history traits to predict viral diversity, host range, and spillover potential, leading to a recent first author paper in *Nature*.

1. [redacted] (b) (4)
2. Memish ZA, Mishra N, Olival KJ, Fagbo SF, Kapoor V, Epstein JH, AlHakeem R, Al Asmari M, Islam A, Kapoor A, Briese T, Daszak P, Al Rabeeah AA, Lipkin WI. (2013). Middle East Respiratory Syndrome Coronavirus in Bats, Saudi Arabia. **Emerging Infectious Diseases**. 19(11): 1819-1823.
3. Olival KJ*, Hosseini P, Zambra-Torrellio C, Ross N, Bogich T, Daszak P*. (2017). Host and viral traits predict zoonotic spillover from mammals. **Nature** 546(7660): 646-650.

*corresponding author

B. Positions and Honors

Positions and Employment

1999 - 02	Research Associate, Kewalo Marine Laboratory, University of Hawaii
2003 - 07	US Environmental Protection Agency STAR Fellow
2006 - 13	Instructor, Columbia University Secondary School Summer Program
2010 - 15	Senior Research Scientist, EcoHealth Alliance
2015 - 17	Associate Vice President for Research, EcoHealth Alliance
2009 -	Visiting Scientist, American Museum of Natural History
2009 -	Adjunct Faculty, Earth Institute Center for Environmental Sustainability, Columbia University
2017 -	Vice President for Research, EcoHealth Alliance

Other Experience and Professional Memberships

1998 - 00	Member, AAAS
2000 - 02	Mentor, NSF Undergraduate Mentoring in Environmental Biology (UMEB), University of Hawaii
2003 - 05	Member, American Society of Mammalogists
2005 - 06	Member, New York Academy of Sciences
2011 -	Scientific Steering Committee Member, Southeast Asian Bat Conservation Research Unit
2011 -	Scientific Advisory Board Member, Lubee Bat Conservancy, FL
2011 -	Scientific Advisor, Bat Conservation International
2011 -	Review Editor, EcoHealth
2015 -	US White-Nose Syndrome Stakeholder Committee and Communications Committee Member
2015 -	Island and Seas, Board Member
2017 -	DoD DTRA: Steering Committee Member, Bat One Health Research Network

Honors

1993-97	Colorado State University Distinguished Scholar Award
2003	NSF Graduate Student Fellowship, Honorable Mention
2005-07	Bat Conservation International Student Award and Scholarship
2004-07	US EPA STAR Fellowship Award
2008	PhD *with Distinction*, Columbia University
2013	Plenary talk on bat virus modeling at 11th Annual ASM Biodefense and EID Research Meeting
2013-14	Institute of Medicine, Forum on Microbial Threats. Invited speaker, briefings on MERS-CoV and Emerging Viral Diseases
2016	Plenary Speaker, NYC Medtech conference – Global Virome Project
2017-18	Three papers awarded the InCites Highly Cited Paper™ designation (top 1% in field) for Immunology and Microbiology

C. Contribution to Science

1. <u>**Viral Discovery and Characterization in Bats**</u>
   A large body of my research has focused on understanding the distribution and diversity of viruses in wildlife populations to better understand the ecological risk of viral emergence. This includes the first use of species accumulation curves to estimate viral diversity using data from longitudinal surveillance of fruit bats in Bangladesh, and a large meta-analysis of viral prevalence in bats to optimize discovery strategies. Two field studies highlighted below include a broad geographic survey of bat coronaviruses in Thailand, and the first isolation and full genome characterization of Nipah virus from the large flying fox in Malaysia.

   a. Rahman SA, Hassan SS, <u>Olival KJ</u>, Mohamed M, Chang L-Y, Hassan L, Saad NM, Shohaimi SA, Mamat ZC, Naim MS, Epstein JH, Suri AS, Field HE, Daszak P and HERG. (2010). Characterization of Nipah virus from Naturally Infected *Pteropus vampyrus* Bats, Malaysia. **Emerging Infectious Disease** 16(12): 1990-1993.

b. Anthony SJ, Epstein JH, Murray KA, Navarrete-Macias I, Zambrana-Torrelio CM, Solovyov A, Ojeda-Flores R, Arrigo NC, Islam A, Khan SA, Hosseini P, Bogich TL, Olival KJ, Sanchez-Leon MD, Karesh WB, Goldstein T, Luby SP, Morse SS, Mazet JAK, Daszak P, Lipkin WI. (2013). A Strategy To Estimate Unknown Viral Diversity in Mammals. **Mbio.** 4(5): e00598-13.

c. Wacharapluesadee S, Duengkae P, Rodparn A, Kaewpom T, Maneeorn P, Kanchanasaka B, Yinsakmongkon S, Sittidetboripat N, Chareesaen C, Khlangsap N, Pidthong A, Leadprathom K, Ghai S, Epstein JH, Daszak P, Olival KJ, Blair PJ, Callahan MV, Hemachudha T. (2015). Diversity of Coronavirus in Bats from Eastern Thailand. **Virology Journal** 12:57.

d. Young CC and Olival KJ*. (2016). Optimizing Viral Discovery in Bats. **PLOS ONE** 11(2): e0149237.

2. Serological Surveillance

Bats are believed to harbor a unique and large diversity of viruses, including a number of pathogens that pose a risk to human health (e.g. Ebola, Nipah, SARS-CoV). I have been involved with field and laboratory investigations of several bat-borne pathogens that pose the greatest risk to humans over the years, including Filoviruses, Henipaviruses, and SARS and MERS-related Coronaviruses. Collection and analysis of serological data was critical to each of these studies. Using serological and PCR data we discovered that bats are reservoirs of Ebola Reston virus in the Philippines. Extensive, proactive surveillance of wild bat and primate populations in Thailand for Ebola viruses importantly showed that several suspected species are likely *not* important reservoirs. The work in Thailand was predicated by my own investigations in Bangladesh where we discovered the first evidence for Ebola Zaire virus infection in a wildlife species outside of Africa – changing our paradigm as to where these viruses can be found globally. Lastly, I have been involved with extensive work to identify the natural reservoir host of Reston virus in Philippines that included both molecular and serological findings.

a. Olival KJ*, Islam A, Yu M, Anthony SJ, Epstein JH, Khan SA, Khan SU, Crameri G, Wang LF, Lipkin WI, Luby SP, and Daszak P. (2013). Ebolavirus Antibodies in Fruit Bats, Bangladesh. **Emerging Infectious Diseases** 19(2): 270-273.

b. Wacharapluesadee S, Olival KJ, Kanchanasaka B, Duengkae P, Kaewchot S, Srongmongkol P, Ieamsaard G, Maneeorn P, Sittidetboripat N, Kaewpom T, Petcharat S, Yingsakmongkon S, Rollin PE, Towner JS , Hemachudha T. (2015). Surveillance for Ebola Virus in Wildlife, Thailand. **Emerging Infectious Diseases** 21(12): 2271-2273.

c. Jayme S, Yu M, Jong Cd, Olival KJ, Tagtag A, Hughes T, Foord A, Marsh G, Crameri G, Epstein JH, Santos I, Catbagan D, Lim M, Benigno C, Wang L, Daszak P, Field H, Newman S. (2015). Molecular evidence of Ebola Reston virus infection in Philippine bats. **Virology Journal**. 12(1): 107.

d. [redacted] (b) (4)

3. Modeling Disease Emergence and Spillover Risk

I have used my applied ecology background working with analyses of wildlife and their pathogens to develop new models to improve our global understanding of zoonotic spillover and disease circulation. In addition to my previously mentioned *Nature* paper, this includes studies that examined the environmental drivers of bat virus spillover to humans, cross-species transmission among bat species, spatial analysis of emerging zoonotic disease hotspots, and host-specific determinants of fungal infection in bats. These modeling approaches explicitly use data from PCR- and serology-based field studies, combined with an understanding of wildlife biology and ecology, to assess the environmental and demographic drivers of disease transmission -- bridging the gap between field investigations and modeling transmission risk.

a. Brierley L, Vonhof MJ, Olival KJ, Daszak P, Jones KE. (2016). Quantifying global drivers of zoonotic bat viruses: a process-based perspective. **American Naturalist** 187: E53-64
b. Willoughby AR, Phelps K, PREDICT Consortium, Olival KJ*. (2017). “A Comparative Analysis of Viral Richness and Viral Sharing in Cave-Roosting Bats”. **Diversity** 9 (35).
c. Allen T, Murray KA, Zambrana-Torrelio C, Morse SS, Rondinini C, Di Marco M, Breit N, Olival KJ, Daszak P. (2017). Global hotspots and correlates of emerging zoonotic diseases. **Nature Communications**. 8(1124): 1-10
d. Verant ML, Bohuski EA, Richgels KLD, Olival KJ, Epstein JH, and Blehert DS. (2018). Determinants of *Psudogymnoascus destructans* within bat hibernacula: implications for surveillance and management of white-nose syndrome. **Journal of Applied Ecology** 55: 820-829.

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

HDTRA11710064 Olival (PI) 10/02/17-10/01/22
Understanding the Risk of Bat-Borne Zoonotic Disease Emergence in Western Asia
The goal of this project is to characterize pathogen diversity, strengthen zoonotic disease surveillance capacity, and test key hypotheses about the risk of bat-borne zoonotic disease emergence in Western Asia.
Role: PI

R01 AI110964 Daszak (PI) 06/01/14-05/31/19
Understanding Risk of Bat Coronaviruses
The goal of this study is to analyze the risk of coronavirus spillover from bats to humans in Southern China
Role: co-PI

Emerging Pandemic Threat Program, USAID Mazet (PI) 10/01/14-09/30/19
PREDICT 2
The goal of this project is to create and implement a global virus surveillance system in animals and humans and analyze spillover risk.
Role: Modeling and Analytics Coordinator; Country lead for Indonesia, South Sudan, and Thailand.

Completed Research Support

Emerging Pandemic Threat Program, USAID Mazet (PI) 10/01/09-09/30/14
PREDICT
The goal of this project was to conduct zoonotic virus surveillance in wildlife in 20 countries, and modeling hotspots and drivers for disease emergence.
Role: Key Personnel: Modeling Team; Country lead for Thailand and Indonesia

Service Award, US Fish and Wildlife Epstein (PI) 09/01/12-09/30/14
Characterization of Climatic Parameters within Bat Hibernacula, their Influence on Environmental Loads of *Geomyces destructans*, and Implications for the Migration of White-Nose Syndrome in Bats.
The goal of this project was to identify environmental and other factors that influence the progression and severity of White Nose Syndrome in bats.
Role: co-PI

R01 AI079231 Daszak (PI) 09/18/08-08/31/13

Risk of viral emergence from bats
Modeled hotspots for viral diversity and emergence in bats, discovery of new viruses, and in vitro test of infectiousness for novel pathogens.
Role: Key Personnel: led project implementation, study design, and phylogenetic modeling

Endangered Species grant, USGS Russell, Vonhof, and Olival (PI) 06/18/12-06/17/13
Genetic Approaches to Defining Taxonomic and conservation Units for the Hawaiian Hoary Bat
The goal of this project was to determine the phylogenetic position and conservation genetic units for endangered hoary bats.
Role: co-PI

3R01 TW005869-06S1 Daszak (PI) 09/01/09 – 8/31/11
NIH Fogarty Ecology of Infectious Diseases ARRA award
The goal of this project was to conduct Nipah virus surveillance in wild bat populations and use genetic methods to understand viral circulation in Bangladesh.
Role: Fogarty US Global Health Fellow

BIOGRAPHICAL SKETCH

NAME Ralph Steven Baric	POSITION TITLE Co-Investigator
eRA COMMONS USER NAME (b) (6)	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
North Carolina State University, Raleigh, NC	BS	1977	Zoology
North Carolina State University, Raleigh, NC	Ph.D.	1982	Microbiology
University of Southern CA, School of Med, (Los Angeles, CA)	Post-Doc	1986	Microbiology

A. Personal Statement: The Baric laboratory uses genetic, biochemical, molecular and immunologic approaches to study the molecular mechanisms regulating viral evolution, virus immunity, virus-host interactions and vaccine mediated protective immunity using coronaviruses (CoV), noroviruses and flaviviruses (Dengue) as models. SARS-CoV and MERS-CoV are used as models to address fundamental questions in genetics, structure-function analyses, entry and cross species transmission, fidelity regulation, host susceptibility allele mapping, pathogenesis as well as therapeutic design and testing. Synthetic genomics and reverse genetics are used to create a panel of CoV molecular cDNA clones for SARS-CoV, SARS-like bat coronaviruses (SL-CoV), MERS-CoV, several human coronavirus, Dengue 1-4 and Zika virus. The Baric laboratory has developed key animal models of human disease, including SARS-CoV and SL-CoV pathogenesis in young and aged mice, and CRISPR gene edited mice encoding permissive mutations in the murine dipeptidyl peptidase receptor, making the animals permissive for MERS-CoV infection and disease.

The Baric laboratory has longstanding expertise in CoV evolution and emergence, replication, virus-receptor interactions, genetics, animal model development and pathogenesis. Not only has the Baric laboratory made fundamental breakthroughs in all aspects of CoV genetics, biology and immunology, but it has designed, developed and tested small molecule inhibitors and vaccines against emerging CoVs. Our group has collaborated with Drs. Daszak, Shi and Wang on SARSr-CoVs for the past 3 years, and this R01 is a natural development of this collaboration.

***Qualifications by Publication*: : >314 total publications, >120 since 2013, H-index: 84.**
http://www.ncbi.nlm.nih.gov/sites/myncbi/ralph.baric.1/bibliography/40583903/public/?sort=date&direction=ascending.

Key Manuscripts

1. Sheahan TP, Sims AC, Graham RL, Menachery VD, Gralinski LE, Case JB, Leist SR, Pyrc K, Feng JY, Trantcheva I, Bannister R, Park Y, Babusis D, Clarke MO, Mackman RL, Spahn JE, Palmiotti CA, Siegel D, Ray AS, Cihlar T, Jordan R, Denison MR, Baric RS (2017). Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. **Science Translational Medicine**, 9(396). eaal3653. PMC5567817.
2. Scobey T, Yount BL, Sims AC, Donaldson EF, Agnihothram SS, Menachery VD, Graham RL, Swanstrom J, Bove PF, Kim JD, Grego S, Randell SH, Baric RS (2013). Reverse genetics with a full-length infectious cDNA of the Middle East respiratory syndrome coronavirus. **Proceedings of the National Academy of the Sciences**, 110(40):16157-62. PMC3791741.
3. Menachery, VD, Yount, BL, Debbink, K, Agnihothram, S., Gralinski, LE, Plante, JA, Graham, RL, Scobey T, Ge SY, Donaldson EF, Randell SH, Lanzavecchia A, Marasco WA, Shi Z, Baric RS (2015). A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. **Nature Medicine**, Nov 9. doi: 10.1038/nm.3985. [Epub ahead of print]. PMID:26552008.

4. Cockrell AS, Yount BL, Scobey T, Jensen K, Douglas M, Beall A, Tang XC, Marasco WA, Heise MT, Baric RS (2016). A Mouse Model for MERS Coronavirus Induced Severe Respiratory Distress Syndrome. **Nature Microbiology**, 2:16226. PMC5578707.

B. Positions and Honors.

Employment Experience:

1986-92	Assistant Professor, Department of Parasitology and Laboratory Practice and Department of Epidemiology, University of North Carolina (UNC), Chapel Hill, NC
1992-2001	Associate Professor, Departments of Epidemiology and Microbiology & Immunology, UNC Chapel Hill
2001-	Professor, Departments of Epidemiology and Microbiology and Immunology, UNC Chapel Hill

Selected Awards/Honors:

2018	US Natl. Acad. Sci. "China-US Workshop on Challenges of Emerging Infections, Laboratory Safety and Global Health Security, Jan 2018, Galveston, Tx.
2015	US Natl. Acad. Sci./UK Royal Society Workshop: Sackler Scientific Forum on the Trends in Synthetic Biology and Gain of Function and Regulatory Implications, U.K.
2015	US Natl. Acad. Sci. "China-U.S. Workshop on the Challenges of Emerging Infections, Laboratory Safety, and Global Health Security" September 28-30 in Beijing, China
2015	MERS-CoV Stakeholders Workshop, invited panelist, NIH
2014	National Academy of Sciences: Working Group on Risks and Benefits of Gain of Function Research
2005-15	Review Board, *J. Virology*
2008-15	Senior Editor, *Plos Pathogens*
2008	US Natl. Acad. Sci. Working Group: Gene Sequence Methods for Classification of Select Agents
2007-08	Associate Editor, *Plos Pathogens*
2005-09	Permanent Member, NIH VirB Study Section
2003	Finalist/Runner-up, World Technology Award
1989-94	Established Investigator: American Heart Association
1984-86	Harvey Weaver Scholar, National Multiple Sclerosis Society

C. Contributions to Virology: The Baric laboratory has made significant contributions to our understanding of all aspects of CoV biology, including: i) CoV genetics and reverse genetics for SARS-CoV, MHV, MERS-CoV, HCoV NL63, PEDV, TGEV, bat SARS-like CoV (SL-CoV), BtCoV HKU-5 and others, ii) demonstration of proof-reading activities in the CoV genome, iii) identification and characterization of bat SL-CoV with prepandemic potential, iii) coronavirus transcription mechanisms, iv) mechanisms of interferon antagonism and interferon stimulated gene expression control, v) virus host susceptibility allele mapping, vi) epitope mapping of human monoclonal antibodies, vii) identification of broad spectrum human monoclonal antibodies against SARS-CoV and MERS-CoV, viii) mouse models of human disease (MERS-CoV and SARS-CoV), ix) aging and emerging coronavirus vaccine efficacy, and x) live and attenuated vaccine design in young and aged animal models of human disease. The Baric laboratory has also made major contributions to norovirus immunology and flavvirus reverse genetics and the human immune responses after infection.

Some representative major contributions outside and within the CoV field include:

1. [redacted] (b) (4)

2. Gralinski LE, Ferris MT, Aylor DL, Whitmore AC, Green R, Frieman MB, Deming D, Menachery VD, Miller DR, Buus RJ, Bell TA, Churchill GA, Threadgill DW, Katze MG, McMillan L, Valdar W, Heise MT, Pardo-Manuel de Villena F, Baric RS (2015) Genome Wide Identification of SARS-CoV Susceptibility Loci Using the Collaborative Cross. **PLOS Genetics,** 11(10): e1005504. PMID:26452100.

3. Lindesmith L, Moe C, Marionneau S, Ruvoen N, Jiang X, Lindblad L, Stewart P, LePendu J, Baric R (2003). Human susceptibility and resistance to Norwalk virus infection. **Nature Medicine**, 9(5):548-53. PMID:12692541.

4. Lindesmith LC, Donaldson EF, Lobue AD, Cannon JL, Zheng DP, Vinje J, Baric RS (2008). Mechanisms of GII.4 norovirus persistence in human populations. **PLOS Medicine**, 5(2):e31. PMC2235898.

C.1. Coronavirus Pathogenesis and Virus Immunity. Our group has studied the role of virus-immune interactions in coronavirus and other emerging virus pathogenesis mechanisms.

1. Rasmussen AL, Okumura A, Ferris MT, Green R, Feldmann F, Kelly SM, Scott DP, Safronetz D, Haddock E, LaCasse R, Thomas MJ, Sova P, Carter VS, Weiss JM, Miller DR, Shaw GD, Korth MJ, Heise MT, Baric RS, de Villena FP, Feldmann H, Katze MG (2014). Host genetic diversity enables Ebola hemorrhagic fever pathogenesis and resistance. **Science**, 2014 346(6212):987-91. PMC4241145.
2. Gralinski LE, Sheahan TP, Morrison TE, Menachery VD, Jensen K, Leist SR, Whitmore A, Heise MT, Baric RS (2018). Complement Activation Contributes to Severe Acute Respiratory Syndrome Coronavirus Pathogenesis. **mBio**, 9(5). e01753-18. PMC6178621.
3. Menachery VD, Eisfeld AJ, Schäfer A, Josset L, Sims AC, Proll S, Fan S, Li C, Neumann G, Tilton SC, Chang J, Gralinski LE, Long C, Green R, Williams CM, Weiss J, Matzke MM, Webb-Robertson BJ, Schepmoes AA, Shukla AK, Metz TO, Smith RD, Waters KM, Katze MG, Kawaoka Y, Baric RS (2014). Pathogenic influenza viruses and coronaviruses utilize similar and contrasting approaches to control interferon-stimulated gene responses. **mBio**, 5(3): e01174-14. PMC4030454.
4. Graham RL, Becker MM, Eckerle LD, Bolles M, Denison MR, Baric RS (2012). A live, impaired-fidelity coronavirus vaccine protects in an aged, immunocompromised mouse model of lethal disease. **Nature Medicine**, 18(12):1820-6. PMCID: PMC3518599.

C.2. Coronavirus Innate Immunity/Animal Models. The Baric laboratory group has studied CoV host range expansion using experimental evolution and SARS-CoV, MERS-CoV, civet SL-CoV, bat SL-CoV, and bat CoV HKU5 as models. This includes synthetic reconstruction of civet and bat CoV from *in silico* sequence, the first reported recovery of recombinant bat viruses, and characterization of host range phenotypes *in vitro* and *in vivo*. Applications of experimental evolution have focused on molecular mechanisms associated with virus-receptor interactions in viral persistence, virus innate immune interactions, and increased virulence in mice.

1. Agnihothram S, Yount BL, Donaldson EF, Huynh J, Menachery VD, Gralinski LE, Graham RL, Becker MM, Tomar S, Scobey TD, Osswald HL, Whitmore A, Gopal R, Ghosh AK, Mesecar A, Zambon M, Heise M, Denison MR, Baric RS (2014). A mouse model for Betacoronavirus subgroup 2c using a bat coronavirus strain HKU5 variant. **mBio**, 5(2): e00047-14. PMC3977350.
2. Sheahan T, Rockx B, Donaldson E, Corti D, Baric R (2008). Pathways of cross-species transmission of synthetically reconstructed zoonotic severe acute respiratory syndrome coronavirus. **Journal of Virology,** 82(17):8721-32. PMC2519660
3. Becker MM, Graham RL, Donaldson EF, Rockx B, Sims AC, Sheahan T, Pickles RJ, Corti D, Johnston RE, Baric R*, Denison MR* (2008). Synthetic recombinant bat SARS-like coronavirus is infectious in cultured cells and in mice. **Proceedings of the National Academy of the Sciences**, 105(50):19944-9. PMC2588415. (* = co-first authors)
4. Menachery VD, Schäfer A, Burnum-Johnson KE, Mitchell HD, Eisfeld AJ, Walters KB, Nicora CD, Purvine SO, Casey CP, Monroe ME, Weitz KK, Stratton KG, Webb-Robertson BM, Gralinski LE, Metz TO, Smith RD, Waters KM, Sims AC, Kawaoka Y, Baric RS (2018). MERS-CoV and H5N1 influenza virus antagonize antigen presentation by altering the epigenetic landscape. **Proceedings of the National Academy of the Sciences**, 115(5): E1012-E1021. PMID: 29339515.

C.3. Virus Genetic Platforms. The Baric laboratory has pioneered reverse genetic analyses of CoVs and DENVs. Several CoV infectious cDNA clones are available in the lab, including SARS-CoV, MERS-CoV, conventional human and model CoVs, and several bat CoVs with pandemic potential. The availability of these genetic platforms allows for detailed studies into the role of viral genes in pathogenesis, innate immune antiviral immunity, vaccine performance and design, virus-receptor interactions, entry and virus evolution.

1. Yount B, Curtis, K, Fritz L, Hensley L, Jahrling P, Prentice E, Denison M, Geisbert T, Baric RS (2003). Reverse Genetics with a full length infectious cDNA for the SARS Coronavirus. **Proceedings of the National Academy of the Sciences**, 100(22): 12995-13000. PMCID: PMC240733.

2. Rockx B, Sheahan T, Donaldson E, Harkema J, Sims A, Heise M, Pickles R, Cameron M, Kelvin D, Baric R (2007). Synthetic reconstruction of zoonotic and early human severe acute respiratory syndrome coronavirus isolates that produce fatal disease in aged mice. **Journal of Virology** 81(14):7410-23. PMC1933338.
3. Widman DG, Young E, Yount BL, Plante KS, Gallichotte EN, Carbaugh DL, Peck KM, Plante J, Swanstrom J, Heise MT, Lazear HM, Baric RS (2017). A Reverse Genetics Platform that Spans the Zika Virus Family Tree. **mBio**, 8(2): e02014-16. PMC5340872
4. Donaldson EF, Yount B, Sims AC, Burkett S, Pickles RJ, Baric RS (2008). Systematic assembly of a full-length infectious clone of human coronavirus NL63. **Journal of Virology**, 82(23):11948-57. PMC2583659.

C4. Virus Vaccine Design and Antiviral Immunotherapy. Viruses are major causes of morbidity and mortality globally. The Baric laboratory has used structure-guided immunogen design and epitope exchange to build multivalent immunogens to increase vaccine breadth and diagnostic potential.

1. Deming DJ, Sheahan T, Heise M, Yount B, Davis N, Sims A, Suthar M, Whitmore JH, Pickles R, West A, Donaldson E, Curtis K, Johnston, RE, Baric RS (2006). Vaccine efficacy in senescent mice challenged with recombinant SARS-CoV bearing epidemic and zoonotic spike variants. **PLOS Medicine**, 3(12): e525 PMCID: PMC1716185.
2. Tang XC, Agnihothram SS, Jiao Y, Stanhope J, Graham RL, Peterson EC, Avnir Y, Tallarico AS, Sheehan J, Zhu Q, Baric RS, Marasco WA (2014). Identification of human neutralizing antibodies against MERS-CoV and their role in virus adaptive evolution. **Proceedings of the National Academy of the Sciences**,111(19):E2018-26. PMC4024880
3. Lindesmith LC, Ferris MT, Mullan CW, Ferreira J, Debbink K, Swanstrom J, Richardson C, Goodwin RR, Baehner F, Mendelman PM, Bargatze RF, Baric RS (2015). Broad blockade antibody responses in human volunteers after immunization with a multivalent norovirus VLP candidate vaccine: immunological analyses from a phase I clinical trial. **PLOS Medicine**, 12(3):e1001807 PMC4371888.
4. Bolles M, Deming D, Long K, Agnihothram S, Whitmore A, Ferris M, Funkhouser W, Gralinski L, Totura A, Heise M, Baric RS (2011). A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge. **Journal of Virology**, 85(23):12201-15. PMC3209347

D.Research Support.

U19 AI 100625 Baric/Heise (MPI) 09/01/17-08/31/22
Systems Immunogenetics of Biodefense Pathogens in the Collaborative Cross
The Collaborative Cross is a mouse resource for study of complex genetic interactions in diverse populations, to identify novel polymorphic genes regulating immune responses to SARS, influenza and WNV, analyze genetic underpinning of immune phenotypes in mice and humans, and generate panels of genetically defined mice to probe polymorphic gene control of immune responses against a pathogens or other immune stimuli.

R01 AI108197 Denison/Baric (MPI) 05/01/18-04/30/23
Determinants of Coronavirus Fidelity in Replication and Pathogenesis
Experiments in this aim will test the hypothesis that nsp14 functions in maintaining high replication fidelity and viral RNA synthesis are coupled and that targeted engineered mutations across nsp14 alter: a) RNA fidelity outcomes; b) sensitivity to nucleoside mutagens and polymerase inhibitors; c) sensitivity to innate immunity.

HHSN272201000019I-HHSN27200003 Baric (PI) 09/30/17-03/31/24
MERS-CoV Mouse Model for Vaccine & Therapeutic Testing (Task Order A57)
Use generation of transgenic mice and modifications to the MERS-CoV genome to identify a mouse model for MERS-CoV that recapitulates human disease phenotypes for evaluating vaccine platforms and therapeutics.

U19 AI 109680 Whitley (PI) 03/01/14-02/28/19
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease. Role: Co-Investigator

U19 AI 109761 Lipkin (PI) 03/01/14-02/28/19
Diagnostic and Prognostic Biomarkers for Severe Viral Disease
The goal is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease, systemic and enteric diseases following virus infection, including coronaviruses, flaviviruses and noroviruses. Role: Project Leader

R01 AI110700 Baric (PI) 04/20/15-03/31/20
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.

[redacted] (b) (4) Baric (PI) [redacted] (b) (4)
Breadth of Blockade Antibody Responses Following Norovirus Vaccination.
[redacted] (b) (4) and UNC will collaborate to evaluate the breadth of the antibody blockade response following norovirus vaccination in various human volunteer populations.

P01 AI106695 Harris (PI) 07/1/2015-6/30/20
Protective immunity following dengue virus natural infections and vaccination
Project 2: Aravinda deSilva and Ralph S. Baric (Co-PI).
The goal is to identify natural correlates of protective immunity following natural infection and or vaccination. Role: Co-Investigator

R01-AI125198 de Silva (PI) 05/01/16 – 04/30/21
Preclinical assays to predict dengue vaccine efficacy
We use samples from DENV tetravalent Sanofi Pasteur vaccine clinical trials to identify mechanisms and correlates of protective immunity or breakthrough infections in vaccinees. Role: Co-investigator.

R01 1AI132178 Baric/Sheahan(MPI) 08/15/17-8/14/22
Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV.
The goal of this proposal is collaborate with Gilead Inc. and obtain GS-5734 preclinical data for IND development and translational studies, all designed to move the therapeutic into human trials.

[redacted] (b) (4) Breuer (PI) [redacted] (b) (4)
Why do Norovirus pandemics occur and how can we control them?
The program uses hospital and community cohorts of NoV infected individuals to ask fundamental questions into the molecular and evolutionary epidemiology of human NoV infections, focusing on the GII.4 strains, leading to new models of virus emergence and disease prevention. Role: Co-Investigator:

R01 AI 089728 Li (PI) 07/01/16-06/30/21
University of Minnesota/NIAID
Receptor recognition and cell entry of coronaviruses
The program studies receptor usage and cell entry mechanisms of emerging coronaviruses, focused on PEDV, MHV and SARS-like Coronaviruses. Role: Co-Investigator

R21 AI135682 Georgiou (PI) 04/01/18-03/30/20
UT Austin/NIAID
Molecular Analysis of Serum Antibody Constituents in Zika Virus Infection.
The goal of this application is to identify antibodies that make up the serologic repertoire after Zikv infection of naive and DENV preimmune individuals. Role: Co-investigator.

R21 AI137887 Moorman/Heise (MPI) 02/05/18-01/31/20
NIH/NIAID $150,000
Molecular Characterization of Functional RNA Structures in the ZikV genome
The goal of this project is to study the RNA Structure of Zika virus. Proposed studies will identify new viral virulence determinants that can be targeted to generate safer and more effective Zika virus vaccines and therapeutics. Role: Co-Investigator.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Noam Ross	Co-Investigator
eRA COMMONS USER NAME (b) (6)	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
Brown University (US)	BS	05/2006	Environmental Sci.
University of California-Davis, (US)	PhD	09/2015	Ecology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YYYY	FIELD OF STUDY
Brown University, Providence, RI	BS	05/2006	Environmental Science
University of California-Davis, Davis, CA	Ph.D	09/2015	Ecology

A. Personal Statement

The goal of our proposal is to identify and quantify the drivers of bat-borne coronavirus spillover in Southern China, by identifying which host traits, viral characteristics, and human behaviors are associated with the highest risk of CoV exposure. This will require statistical and mathematical modeling approaches that can integrate the separate ecological, evolutionary, and behavioral processes into a robust framework. My background in quantitative disease ecology makes me a natural fit to work on the statistical and mathematical aspects of this project. My research has consisted of developing both statistical and theoretical models for emerging diseases in both plants, mammals, and humans. I have developed dynamic models of diseases such as MERS and Ebola virus in wildlife populations in order to support targeting field surveillance, and applied predictive empirical and mechanistic modeling techniques to the study of Nipah virus emergence and circulation in bats. My statistical work has included analysis of survey-based evidence of new disease emergence in Uganda, global predictive models of anthrax emergence, and large-scale macroecological patterns in host-virus associations which captured previously unmodeled heterogeneity in disease burden. Importantly, this work included the creation of methods and open-source tools for simulating, fitting, and performing optimization using such models, ensuring that I will be able to support the creation of robust and reproducible statistical models this project.

a. Olival KJ, Hosseini PR, Zambrana-Torrelio C, Ross N, Bogich TL, Daszak P (2017). Host and viral traits predict zoonotic spillover from mammals. **Nature** 546: 646–650
b. Salerno J, Ross N, Ghai R, Mahero M, Travis DA, Gillespie TR, Hartter J (2017) Human-wildlife interactions predict febrile illness in park landscapes of western Uganda. **EcoHealth** 14(4):675-690.
c. Carlson CJ, Kracalik I, Ross N, Alexander K, Hugh-Jones ME, Fegan M, Elkin B, Epp T, Shury T, Bagirova M, Getz WM, Blackbum JK (2018) The global distribution of *Bacillus anthracis* and associated anthrax risk to humans, livestock, and wildlife. **Nature Microbiology** In Review.

B. Positions and Honors

Positions and Employment

2006 Contract Market Researcher: Energy Efficient Products Initiative, Wal-Mart, Providence, RI
2006 - 07 Analyst, Environmental Markets and Performance, GreenOrder, New York, NY

2007 - 09 Senior Analyst, Environmental Markets and Performance, GreenOrder, New York, NY
2010 - 15 Graduate Researcher, University of California-Davis
2015 - 17 Disease Ecologist, EcoHealth Alliance, New York, NY
2017 - Senior Research Scientist, EcoHealth Alliance, New York, NY

Other Experience and Professional Memberships

2012 - 13 Member, NSF IGERT.org advisory board
2012 - 15 Founder and Organizer, Davis R Users' Group
2013 - Member, Ecological Society of America
2014 - Contributor and reviewer, ROpenSci
2014 - Meeting Session Organizer, Ecological Society of America
2015 - Instructor, Software Carpentry Foundation
2015 - Instructor, Data Carpentry Foundation
2015 - Associate Editor, ROpenSci
2016 - Member, R Epidemics Consortium
Reviewer: *Ecology Letters*, *Theoretical Ecology*, *EcoHealth*, *Conservation Letters, Biological Reviews, Journal of Open Source Software*

Awards and Fellowships

2010 NSF IGERT Traineeship in Rapid Environmental Change
2010 UC Davis Graduate Ecology Fellowship
2012 Don Dahlsten Memorial Grant, California Forest Pest Council
2012 NSF IGERT Bridge Fellowship

C. Contribution to Science

1. **Modeling Dynamics of Heterogeneity:** I have worked on both theoretical and applied approaches of dealing with heterogeneity when modeling ecological-epidemiological dynamics. This work focused on fungal disease epidemics using a framework traditionally used for parasites of stable populations in order to capture the role of individual variation in infection level. While the mathematical basis of these models for populations at or approximately at equilibrium is well established, their dynamic properties are less well known due to analytical intractability, and this they are little-used in emerging diseases and epidemics. My work showed how and where these models diverged from other, traditional models in their dynamical properties, and identified statistical patterns that could be used to identify where these models are appropriate. I developed numerical tools for their simulation, modeling and control, which have been used in applied disease management studies.

    a. Schreiber S, Ross N (2016) Individual-based Integral Projection Models: The role of size-structure on extinction risk and establishment success. **Methods in Ecology and Evolution.** http://dx.doi.org/10.1111/2041-210X.12537

    b. Cobb RC, Ross N, Hayden JK, Eyre CA, Dodd RS, Frankel SJ, Garbelloto M, Rizzo DM (2018) Promise and pitfalls of endemic resistance when cultural resources are threatened by exotic tree pathogens. **Phytopathology.** https://doi.org/10.1094/PHYTO-04-18-0142-R

    c. Cobb RC, Hartsough P, Ross N, Klein J, LaFever DH, Frankel SJ, Rizzo DM (2017) Resiliency or restoration: management of sudden oak death before and after outbreak. **Forest Phytophthoras**. https://doi.org/10.5399/osu/fp.7.1.4021

    d. Ross N (2015). Disease with Multiple Infections: Population Structure, Dynamics, and Control. **University of California, Davis**. Dissertation.

2. **Modeling decision-making in complex systems:** A long-standing theme of my work has been linking ecological dynamics to social systems and decision-making under uncertainty. This has included determining whether statistical signals of ecological changes are sufficient to justify management changes in fisheries, and has recently extended to optimizing investment in disease surveillance and intervention.

a) Machalaba C, Smith KM, Awada L, Berry K, Berthe F, Bouley TA, Bruce M, Abrahantes JC, Turabi EL, Feferholtz Y, Flynn L, Fournié G, Andre A, Grace D, Jonas O, Kimani T, Gall FL, Jose J, Peyre MM, Pinto J, Ross N, Rüegg SR, Salerno RH, Seifman R, Zambrana-Torrelio C, Karesh WB. (2017) One Health Economics to confront disease threats. **Transactions of the Royal Society of Tropical Medicine and Hygiene** https://doi.org/10.1093/trstmh/trx039

b) Boettiger C*, Ross N*, Hastings A (2013) Early Warning Signals: The Charted And Uncharted Territories. **Theoretical Ecology** http://dx.doi.org/10.1007/s12080-013-0192-6 (*Co-equal authors)

c) Fuller K, Kling D, Kroetz K, Ross N, Sanchirico JN (2013) Economics and Ecology of Open-Access Fisheries. In: Shogren JF (ed.) **Encyclopedia of Energy, Natural Resource, and Environmental Economics, Vol. 2** p.39-49. Amsterdam: Elsevier. http://dx.doi.org/10.1016/B978-0-12-375067-9.00114-5

3. **Statistical software and reproducibility:** As associate editor of the ROpenSci project, and a member of the Software Carpentry foundation, I develop, evaluate, and set standards and develop training materials for open-source statistical software overseeing the publication of over 30 scientific software packages in the past two years. I have also worked in the development and dissemination of tools for the use of nonlinear modeling methods.

   a. Ross N (2016) fasterize: high performance raster conversion for modern spatial data. https://github.com/ecohealthalliance/fasterize

   b. [redacted] (b) (4)

   c. [redacted] (b) (4)

   d. Ross N (2018) Nonlinear Modeling in R with GAMs: An Interactive Course. **DataCamp** https://www.datacamp.com/courses/nonlinear-modeling-in-r-with-gams

D. Research Support

Ongoing

USAID EPT PREDICT-2 Mazet (PI) 10/01/14 – 09/30/19
Conducting surveillance for novel pathogens in wildlife, livestock and people; characterizing human risk behavior; modeling risk of novel disease emergence; identifying mitigation strategies
Amount: $35 Million subcontract from a $100 Million award
Role: Disease Ecologist

1R01AI110964 Daszak (PI) 06/01/14 – 05/31/19
NIAID: Understanding the Risk of Bat Coronavirus Emergence
Bat ecological, human risk behavioral and virological studies to understand the risk of bat coronavirus emergence
Role: Key Personnel

HDTRA1-14-1-0029 Karesh (PI) 5/17/16 – 5/16/18
Understanding Rift Valley Fever in Republic of South Africa
Role: Key Personnel

Completed

W911NF-13-1-0305 Hastings (PI) 9/1/13-8/31/16
Army Research Office Mathematical Sciences Core Program
Dynamics at Intermediate Time Scales and Management of Ecological Populations

Role: Supported Graduate Student

EF-0622770 Rizzo (PI) 8/23/06-8/31/11
NSF Ecology of Infectious Disease Program
Collaborative Research: Sudden Oak Death: Feedback Between a Generalist Pathogen, Hosts, and Heterogeneous Environments at Multiple Spatial and Temporal Scales
Role: Supported Graduate Student

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Alice Latinne	Research Scientist
eRA COMMONS USER NAME (b) (6)	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
University of Namur, Namur, Belgium	BSC	06/2004	Biology
University of Liege, Liege, Belgium	MSC	06/2006	Animal Biology
University of Liege, Liege, Belgium	PHD	12/2012	Molecular Biology

A. Personal Statement

My research focuses on understanding the dynamics of pathogens within and among wildlife populations, livestock, and humans. I have conducted fieldwork in Asia for the past 6 years, focused on the evolutionary dynamics of host-pathogen (rodent-virus; bat-virus) interactions, the phylogenetics of co-evolution, and analysis of phylogeographic scale. My main interest is to analyze he risk of zoonotic pathogen emergence at high-risk human-wildlife interfaces. My published work analyzes patterns and likelihood of pathogen sharing among species, and to determine how the host phylogenetic and phylogeographic structure affects pathogen distribution and cross-species transmission. Prior to my current position at EcoHealth Alliance, I was a Marie Curie COFUND fellow conducting postdoctoral research at the Institut des Sciences de l'Evolution in Montpellier (ISEM, France) and at the Kasetsart University in Thailand.

B. Positions and Honors

Positions and Employment

2013-2013 Research Assistant, University of Liege, Liege, Belgium
2014- Research Associate, University of Liege, Liege, Belgium
2015- Research Scientist, EcoHealth Alliance, New York

Honors

2007 Belgian Government graduate scholarship, Belgian Fund for Research in Industry and Agriculture, Belgium
2008 Belgian Government graduate scholarship, Belgian Fund for Scientific Research, Belgium
2013 Award "VOCATIO" (Vocation grant) from the Belgian Foundation of Vocation (VOCATIO)
2013 Marie Curie COFUND fellowship from European Union

C. Contribution to Science: Selected peer-reviewed publications most relevant to the current application

1. Latinne A, Bezé F, Delhaes L, Pottier M, Gantois N, Nguyen J, Blasdell K, Dei-Cas E, Morand S, Chabé M (2017). Genetic diversity and evolution of *Pneumocystis* fungi infecting wild Southeast Asian murid rodents. **Parasitology**, 145(7): 885-900. PMID: 29117878

2. Olival KJ, Latinne A, Islam A, Engstrand R, Hersch R, Amato G, Epstein JH, Daszak P (2016). Using bat population genetics to understand Nipah virus dynamics and cross-species transmission in south and southeast Asia. **International Bat Research Conference**, Durban.

3. Morand S, Bordes F, Chen H, Claude J, Cosson J, Galan M, Czirjak GA, Greenwood A D, Latinne A, Michaux J, Ribas A (2015) Global parasite and *Rattus* rodent invasions: the consequences for rodent-borne diseases. **Integrative Zoology**, 10(5), 409-423. PMID: 26037785

4. Latinne A, Meynard CN, Herbreteau V, Waengsothorn S, Morand S, Michaux J (2015). Influence of past and future climate changes on the distribution of three Southeast Asian murine rodents. **Journal of Biogeography**, 42(9), 1714-1726. doi.org/10.1111/jbi.12528

5. Blasdell K, Bordes F, Chaval Y, Claude J, Cosson J, Latinne A, Michaux J, Morand S, Pagès M, Tran A (2015). Progress on research on rodents and rodent-borne zoonoses in South-east Asia. **Wildlife Research**, 42(2), 98-107. doi.org/10.1071/WR14201

Additional recent publications

1. Mouton A, Mortelliti A, Grill A, Sara M, Kryštufek B, Juškaitis R, Latinne A, Amori G, Randi E, Büchner S, Schulz B, Ehlers S, Lang J, Adamik P, Verbeylen G, Dorenbosch M, Trout R, Elmeros M, Aloise G, Mazzoti S, Matur F, Poitevin F, Michaux JR (2017). Evolutionary history and species delimitations: a case study of the hazel dormouse, *Muscardinus avellanarius*. **Conservation Genetics**, 18(1): 181-196. doi.org/10.1007/s10592-016-0892-8

2. Smitz N, Cornélis D, Chardonnet P, Caron A, de Garine-Wichatitsky M, Jori F, Mouton A, Latinne A, Pigneur L, Melletti M, Kanapeckas KL, Marescaux J, Lopes-Pereira C, Michaux J (2014). Genetic structure of fragmented southern populations of African Cape buffalo (*Syncerus caffer caffer*). **BMC Evolutionary Biology**, 14: 203. doi.org/10.1186/s12862-014-0203-2

3. Latinne A, Galan M, Waengsothorn S, Rojanadilok P, Eiamampai K, Sribuarod K, Michaux J (2014). Diet analysis of *Leopoldamys neilli*, a cave-dwelling rodent in Southeast Asia, using Next-Generation Sequencing from feces. **Journal of Cave and Karst Studies**, 76(2): 139-145. doi.org/10.4311/2013LSC0100

4. Latinne A, Chaval Y, Waengsothorn S, Rojanadilok P, Eiamampai K, Sribuarod K, Herbreteau V, Morand S, Michaux J (2013). Is *Leopoldamys neilli* (Rodentia, Muridae) a synonym of *Leopoldamys herberti*? A reply to Balakirev *et al.* (2013). **Zootaxa**, 3731(4): 589-598. doi.org/10.11646/zootaxa.3731.4.10

5. Latinne A, Waengsothorn S, Rojanadilok P, Eiamampai K, Sribuarod K, Michaux J (2013). Diversity and endemism of Murinae rodents in Thai limestone karsts. **Systematics and Biodiversity**, 11(3): 323-344. doi.org/10.1080/14772000.2013.818587

6. Pauwels OSG, Sumontha M, Latinne A, Grismer LL (2013). *Cyrtodactylus sanook* (Squamata: Gekkonidae), a new cave-dwelling gecko from Chumphon Province, southern Thailand. **Zootaxa**, 3635(3): 275-285. PMID: 26097949

7. Latinne A, Waengsothorn S, Rojanadilok P, Eiamampai K, Sribuarod K, Michaux J (2012). Combined Mitochondrial and Nuclear Markers Revealed a Deep Vicariant History for *Leopoldamys neilli*, a Cave-Dwelling Rodent of Thailand. **PLOS One**, 7(10), e47670. PMID: 23118888

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Hongying Li	Research Scientist
eRA COMMONS USER NAME (b) (6)	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
School of Life Sciences, Sun Yat-Sen University, China	BS	06/2012	Biosciences
School of Public Health, Emory University	MPH	05/2015	Health Policy
School of Life Sciences, Kingston University, UK	Ph.D Candidate	2018-	Infectious Diseases

A. Personal Statement

I have an interdisciplinary background in ecology, public health, and human behavior, coupled with extensive on-the-ground experience working with communities, governmental and academic partners in China. For the past 3 years I have worked as China Programs Coordinator at EcoHealth Alliance, acting as the key point-of-contact among EcoHealth staff and our partners in China. I have coordinated fieldwork to conduct bat sampling, and human behavioral risk assessments across 5 provinces in southern China. I have also liaised directly with all key partners on this proposal. Additionally, I coordinate EcoHealth Alliance's wildlife trade research in China and SE Asia focusing on analyzing incentives to trade and consume wildlife. I work closely with Chinese Health and Forestry governmental departments, research institutes, and local organizations to foster collaboration and communication as part of my PhD research on *"Policy and Human Behavioral Strategies to Mitigate Zoonotic Disease Emergence in Southern China".*

B. Positions and Honors.

Positions and Employment

2011 - 12 Research Assistant of HIV Prevention Program, Yunnan Maternity and Children's Hospital, China
2013 - 14 Program Assistant of School HIV/AIDS & School Education, UNESCO Beijing, China
2015 - China Programs Coordinator & Research Scientist, EcoHealth Alliance, USA
2017 - Coordinator of the Initiative of National Virome Project in China

Other Experience and Professional Memberships

2018- Member, IUCN SSC Pangolin Specialist Group
2018- Member, Society for Applied Microbiology
2017- Member, China Health Policy and Management Society
2016- Member, International Association for Ecology & Health
2016- Columnist, *China Environment*
2016- Asian Representative, Conservation Leadership Programme

Honors

2010 National Scholarship, Ministry of Education, the People's Republic of China.
2012 Outstanding Graduate Award, Sun Yat-sen University, China
2016 Invited speaker, China Conservation Network workshop. "Impacts of wildlife trade on public health"
2017 Invited Speaker, International Association for Ecology & Health. "Understanding the wildlife trade in China"

C. Selected peer-reviewed publications most relevant to the current application

Liang X, Zhang L, Wan Y, Yu X, Guo Y, Chen X, Li H (2012). Changes in the diurnal rhythms during a 45-day head-down bed rest. **PLOS One**, 7(10), e47984.

Wu Z, Lu L, Du J, Yang L, Ren X, Liu B, Li H, Zhu Y (2018). Comparative analysis of rodent and small mammal viromes to better understand the wildlife origin of emerging infectious diseases. **Microbiome**, 6(1), 178.

Additional recent publications of importance to the field (in chronological order)

Li H, Zhu G, Zhang Y, Daszak P (2018). Qualitative Approach to Developing a One Health Intervention Strategy for Zoonosis Risk Mitigation in Southern China. Poster Presentation at **One Health Congress 2018**.

Li H, Chmura AA, Ma C, Gabriel G, Daszak P (2018). Attitudes Towards Wildlife Trade and Disease Risk in China. Poster presentation at **One Health Congress 2018**.

Li H, Zhu G, Zhang Y, Daszak P (2018). Viral Pathogen Discovery in China: Understanding the Risks of Bat Coronaviruses. Poster presentation at **USAID EPT-2 PREDICT Meeting**.

D. Research Support

Ongoing Research Support

R01 AI110964 Daszak (PI) 06/01/14-05/31/19
Understanding Risk of Bat Coronaviruses
The goal of this study is to analyze the risk of coronavirus spillover from bats to humans in Southern China
Role: Project Coordinator & Human Research Lead

Emerging Pandemic Threat Program, USAID Mazet (PI) 10/01/14-09/30/19
PREDICT 2
The goal of this project is to create and implement a global virus surveillance system in animals and humans and analyze spillover risk.
Role: Country Coordinator for China

Completed Research Support

[redacted] (b) (4) Zhang (PI) 01/01/16-12/31/17
The goal of this study is to understand the current population and distribution of the critically endangered Chinese pangolin *(Manis pentadactyle)* in mainland China
Role: Community Research Lead

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Leilani V. Francisco	Co-Investigator
eRA COMMONS USER NAME [redacted] (b) (6)	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
University of Maryland, College Park, Maryland	BA	05/1995	Anthropology (Sociocultural)
University of South Florida, Tampa, Florida	MA	05/2002	Applied Anthropology (Medical)
Johns Hopkins University, Baltimore, Maryland	Ph.D	05/2010	Public Health (Int'l Health)
Project Management Institute, Pennsylvania	PMP	Current	Project Management

A. Personal Statement

I have a Bachelors and Masters degree in anthropology and applied anthropology, and over 20 years of professional experience managing human behavioral research projects in public health, the majority of which has been in developing countries. I have extensive experience in the private sector, managing research projects and evaluating public health interventions for infectious diseases. I have worked extensively on: quantitative, qualitative, and mixed-methods study design, data collection, and analysis; management of behavioral intervention projects, public health assessments, and behavioral research study design. My work has focused on HIV/AIDS and other zoonotic infectious diseases, and sociocultural behavior change interventions. Previously, I managed a portfolio of global health contracts valued at over $20 million in service to the U.S. Agency for International Development (USAID), [redacted] (b) (4), and the Centers for Disease Control and Prevention (CDC). While my private sector career meant that I was not able to publish much of my work, I generated over 80 high-profile technical reports for federal and international health agencies. At EcoHealth Alliance I lead a behavioral risk team for USAID/EPT PREDICT (project ending 2019) characterizing behavioral risk in 28 countries with high-risk human-animal disease transmission interfaces. I have been [redacted] (b) (4)

B. Positions and Honors

Positions and Employment

2017-Present	Senior Scientist, EcoHealth Alliance, NY
2017-Present	USAID PREDICT-2 Global Director for Behavioral Risk Surveillance, NY
2017-Present	USAID PREDICT-2 Partner Lead for Ecological and Biological Human Surveillance, NY
2013-2017	Lead Associate / Senior Lead Scientist, Booz Allen Hamilton, Washington, DC
2010-2012	Associate / Lead Scientist, Booz Allen Hamilton, Washington, DC
2010	Research Consultant, Johns Hopkins Bloomberg School of Public Health, Center for Communication Programs, Baltimore, MD
2010	Research Consultant, Academy for Educational Development, Washington, DC
2007-2008	Research Fellow in Social Epidemiology, London School of Hygiene and Tropical Medicine, London, UK and Kampala, Uganda
2007-2008	SASA! Study Baseline Project Leader, London School of Hygiene and Tropical Medicine, London, UK and Kampala, Uganda
2005-2007	Senior Research Analyst, American Institutes for Research, Washington, DC
2004-2005	Research Analyst, American Institute for Research, Washington, DC
2003	Research Consultant, International Center for Research on Women, Washington, DC
2002-2004	Health Research Scientist, Battelle Memorial Institute, Arlington, VA

1999-2001	Health Researcher, Battelle Memorial Institute, Arlington, VA
1998-1999	Graduate RA, Center for Urban Transportation Research, Tampa, FL
1998	Graduate RA, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL
1997-1998	Graduate RA, University of South Florida, Department of Anthropology, Tampa, FL
1996-1997	Project Manager, Cultural Systems Analysis Group, Univ Maryland, College Park, Maryland
1995-1996	RA, Cultural Systems Analysis Group, University of Maryland, College Park, Maryland

Other Experience and Professional Memberships

Member, American Public Health Association (APHA)
Member, American Evaluation Association (AEA)
Member, Global Health Council (GHC)
Member, American Anthropological Association (AAA)
Member, Society for Applied Anthropology (SfAA)

Honors

2007-2010	Johns Hopkins University Tuition Scholarship
2003	Distinguished Service Award, Latin American Youth Center
1999	Center for Urban Transportation Research Graduate Assistantship
1999	Latin American and Caribbean Studies Passport Scholarship
1998	Latin American and Caribbean Studies Research Grant
1997-1998	Department of Anthropology Graduate Assistantship

C. Contribution to Science

1. **Ethical and Robust Human Subjects Research**: My advanced training and experience in designing, carrying out, and evaluating mixed-methods research projects with vulnerable human populations, has allowed me to contribute to the body of literature and recommended practices around balancing robust study design with the ethical treatment of human subjects. With public health research and evaluation experience spanning Africa, Asia, Central America, the Caribbean, and North America, my contributions within this subject area have added to the discourse of building, implementing, and measuring scientific exploration in the name of human health improvements without compromising human privacy, dignity, and respect.

    a. Francisco LV, Abramsky T, Kiss L Michau L, Musuya T, Kerrigan D, Kaye D, Watts C (2013). Violence against Women and HIV Risk Behaviours in Kampala, Uganda: Baseline Findings from the SASA! Study. **Violence Against Women**, 19(7): 814-832.

    b. Wagman J, Francisco LV, Glass N, Sharps PW, Campbell JC (2008). Ethical challenges of research on and care for victims of intimate partner violence. **Journal of Clinical Ethics**,19(4):371-80.

    c. Campbell JC, Baty ML, Ghandour RM, Stockman JK, Francisco LV, Wagman J (2008). The intersection of intimate partner violence against women and HIV/AIDS: a review. **International Journal of Injury Control and Safety Promotion**, 15(4), 221-31.

    d. Campbell, JC, Baty ML, Ghandour RM, Stockman JK, Francisco LV, Wagman J (2008). The Intersection of Violence against Women and HIV/AIDS. In Scott KA (Rapporteur) **Violence Prevention in Low- and Middle- Income Countries: Finding a Place on the Global Agenda**, pp.149-166. Washington, DC: Institute of Medicine, National Academies Press.

2. **Scientific approaches to behavioral intervention**: Through the example of my work as a scientist with subject matter expertise in behavior change, I have built a strong case that scientific evidence can and should make its way into the hands of decision-makers and the community. This evidence-action gap is one that is often recognized, but regularly left unaddressed. My work in Kampala Uganda using a cluster randomized controlled trial to understand the impact of an intervention in preventing violence against

women and reducing their HIV risk was recognized by Harvard University as a program that closes gender gaps in economic opportunity, politics, health, and education. It was also added to the Women and Public Policy Program's Gender Action Portal, a hub of scientific evidence providing insights on the impact of policies, strategies and practices aimed at closing gender gaps, and taking promising interventions to scale. Additionally, I led the development of a behavioral intervention resource in the form of a moderated picture book, "Living Safely with Bats," based upon feedback from communities living in countries and in areas of regular bat-human contact in their homes. This resource became a key component in ministerial and community outreach by the USAID PREDICT consortium following the announcement of the discovery of the Bombali ebolavirus in 2018, and reflects my continued efforts to translate research to practice.

a. Abramsky T, Devries K, Kiss L, Nakuti J, Kyegombe N, Starmann E, Cundill B, Francisco LV, Kaye D, Musuya T, Michau L, Watts C (2014). Findings from the SASA! Study: a cluster randomised controlled trial to assess the impact of a community mobilisation intervention to prevent violence against women and reduce HIV risk in Kampala, Uganda. **BMC Medicine**, 12:122.

b. Francisco LV, Sullivan A, Goley J, Martinez S, Saylors K, Euren J, Epstein JH, Bird B, Goldstein T, Wolking D, Johnson C, Hagan E, Olival KJ, Karesh WB, Daszak P, Mazet JK (2018). Living Safely with Bats: a risk-reduction resource to help communities in developing countries change behavior to minimize zoonotic spillover from bats. **USAID** Washington, DC.

c. Campbell JC, Baty ML, Ghandour RM, Stockman JK, Francisco LV, Wagman J (2008). The Intersection of Violence against Women and HIV/AIDS. In Scott KA (Rapporteur). **Violence Prevention in Low- and Middle- Income Countries: Finding a Place on the Global Agenda**, pp.149-166. Washington, DC: Institute of Medicine, The National Academies Press.

3. **Applied behavioral research**: Through my advanced training and experience in quantitative, qualitative, and mixed-methods research methodology I have focused on promoting the application of behavioral research to on-the-ground problems. My authorship of over 80 technical reports and publications reinforces my track record of commitment to making robustly-generated methodologies available and accessible to those who affect policy and programming.

a. Francisco LV, *et al.* (2015). DTRA CBEP Country Assessment Manual: Guidance for Implementation of CBEP Assessments of Country Capabilities in Biosurveillance, Biosafety, and Biosecurity. **Booz Allen Hamilton**, Lorton, VA.

b. Francisco LV, *et al.* (2011). Resilience and Prevention Study: Program Evaluation Framework for the Never Leave a Marine Behind (NLMB) Program. For the Defense Centers of Excellence for Psychological Health and Traumatic Brain Injury, US Department of Defense. **Booz Allen Hamilton**, Rockville, MD.

c. Francisco LV (2010). Operational Plan for Ethnographic and Network Assessment Research Project. For Centers for Disease Control and Prevention HIV prevention project in Côte d'Ivoire and Zambia. **Academy for Educational Development**, Washington, DC.

Complete List of Published Work in MyBibliography:

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01 AI110964 Daszak (PI) 06/01/14-05/31/19
NIAID: Understanding the Risk of Bat Coronavirus Emergence
Bat ecological, human risk behavioral and virological studies to understand the risk of bat coronavirus emergence
Role: Research Scientist

USAID EPT PREDICT-2 Mazet (PI) 10/01/14 – 09/30/19

Conducting surveillance for novel pathogens in wildlife, livestock and people; characterizing human risk behavior; modeling risk of novel disease emergence; identifying mitigation strategies
The goal of this project is to assist focal countries in monitoring viruses with pandemic potential, as well as the behaviors, practices, and conditions that are associated with viral evolution, spillover, amplification, and spread.
Role: Research Scientist

Completed Research Support

CDC CGH DGHT Zambia ART Bell (Project Director) 03/28/16 – 01/15/17
Centers for Disease Control and Prevention (CDC), Center for Global Health (CGH), Division of Global HIV/AIDS and Tuberculosis (DGHT), ART Readiness in HIV-infected Pregnant Women: From Formative Qualitative Research to Individual Randomized Trial – Zambia
Trial monitoring visits to evaluate accuracy of screening instrument and effectiveness of enhanced adherence package through early data on virologic response, mother to child transmission (MTCT) rates of HIV, and renal function.
Role: Project Manager

PFSCM Projects McLaughlin (Officer in Charge) 06/01/2014 – 01/15/17
Partnership for Supply Chain Management (PFSCM) Projects: USAID Supply Chain Management System (SCMS); Global Fund Pooled Procurement Mechanism (PPM); 3MDG Regional Supply Chain Strengthening (RSCS)
Led and oversaw all company-wide team members and activities associated with these three projects, as part of a 16-member consortium, known as the Partnership for Supply Chain Management (PFSCM). All projects focused on increasing regular and consistent HIV/AIDS treatment through health systems strengthening, performance management, country strategic planning, and technical assistance provision.
Role: Program Manager

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Amy Catherine Sims	Co-Investigator
eRA COMMONS USER NAME (b) (4)	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
University of Alabama at Birmingham	BS	05/1995	Molecular Biology
Vanderbilt University, Nashville, TN	PhD	05/2001	Microbiology & Immuno
Duke University, Durham, NC	Postdoctoral	08/2002	RNA/Protein Interaction
University of North Carolina at Chapel Hill (US)	Postdoctoral	10/2005	Virology

A. Personal Statement

The identification of highly pathogenic human coronaviruses (SARS-CoV and MERS-CoV) underscored the importance of understanding how viruses emerge from zoonotic reservoirs and how these emergent viruses replicate and cause pathogenesis in the new host. My research has focused on several key aspects of these questions by working to understand the cellular tropism of SARS-CoV and MERS-CoV in primary human lung cells, how host genetic pathways and gene networks affect virus replication and pathogenesis and how manipulating the coronavirus genome changes the host innate immune response to virus infection.Dr. Sims created the humanized transgenic mice that facilitate bat coronavirus replication in coronavirus small animal models and has significant expertise using the coronavirus reverse genetics platform established at UNC.She pioneered the use of primary human lung cell cultures for understanding coronavirus cellular permissivity, in vitro replication kinetics, and therapeutic treatment options within the Baric laboratory.

Relevant publications: My most relevant work to date focuses on using primary human lung cells as culture models for human and human-like bat coronavirus strains.

1. Menachery VD, Yount BL, Sims AC, Agnihothram S, Gralinski LE, Plante JA, Graham RL, Scobey T, Royal S, Pickles RJ, Randell SH, Lanzavecchia A, Marasco WA, Shi Z, Baric RS (2016). SARS-like WIV1-CoV poised for human emergence. **Proceedings of the National Academy of the Sciences** 15:113(11): 3048-53. PMC4801244
2. Becker MM, Graham RL, Donaldson EF, Rockx B, Sims AC, Timothy Sheahan, Raymond Pickles, Davide Corti, Robert E. Johnston, Ralph S. Baric, Mark R. Denison (2008). Platforms for the Synthetic Reconstitution of Noncultivable Zoonotic Viruses. **Proceedings of the National Academy of the Sciences** PMC2588415
3. Sims AC, Baric RS, Yount B, Burkett SE, Jeffers L, Pickles RJ (2005). SARS-CoV infection of human ciliated airway epithelium: the role of the ciliated cell in viral spread in the conducting airways of the lung. **Journal of Virology** 79(24):15511-15524, 2005. PMC1316022
4. Scobey T, Yount BL, Sims AC, Donaldson EF, Agnihothram SS, Menachery VD, Graham RL, Swanstrom J, Bove PF, Kim JD, Grego S, Randell SH, Baric RS. Reverse genetics with a full-length infectious cDNA of the Middle East respiratory syndrome coronavirus.**Proceedings of the National Academy of the Sciences** U S A. 2013 Oct 1;110(40):16157- 62. PMID: 24043791. PMC3791741
5. Sims AC, Sheahan TP, Graham RL, Menachery VD, Gralinski LE, Case JB, Leist SR, Pyrc K, Feng JY, Trantcheva I, Bannister R, Park Y, Babusis D, Clarke MO, Mackman RL, Siegel D, Ray AS, Cihlar T, Jordan R, Denison MR, Baric RS (2017). Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. **Science Translational Medicine** 28;9(396). PMC5567817

B. Positions and Honors

1993 American Society of Microbiology Undergraduate Research Award, University of Alabama
1994 Albert Einstein College of Medicine Summer Student Award
1996 - 01 Graduate Student, Laboratory of Mark Denison, Vanderbilt University, Nashville, TN
1999 Dissertation Enhancement Award, Vanderbilt University
2001 - 02 Postdoctoral Fellow, Laboratory of Jack Keene, Duke University, Durham, NC
2002 - 05 Postdoctoral Fellow, Laboratory of Ralph Baric, UNC at Chapel Hill
2002 - 04 Infectious Disease Pathogenesis Training Grant Fellow (NIH/NIAID 5T32AI07151-27)
2005 - 17 Research Assistant Professor, Department of Epidemiology, UNC, Chapel Hill, NC
2017 - Research Associate Professor, Department of Epidemiology, UNC, Chapel Hill, NC

C. Contributions to Science

1. *In vitro* models for viral infection. Finding suitable in vitro models for studying newly identified or emerged human respiratory viruses can be a challenge. Primary cells isolated from the human conducting airway can be cultured at an air liquid interface and following maturation recapitulate the morphology of the airway epithelium. These cultures provide a unique in vitro model and for one human coronavirus, HKU1, provide the only in vitro model for studying this virus.

a) Sims AC, Pyrc K, Dijkman R, Jebbink M, Long C, Deming D, Donaldson E, Vabret A, Baric RS, van der Hoek L, Pickles R (2010). Culturing the unculturable: human coronavirus HKU1 infects, replicates, and produces progeny virions in human ciliated airway epithelial cell cultures. **Journal of Virology**, 84(21): 11255-63. PMC2953148

b) Sims AC, Baric RS, Yount B, Burkett SE, Jeffers L, Pickles RJ (2005). SARS-CoV infection of human ciliated airway epithelium: the role of the ciliated cell in viral spread in the conducting airways of the lung. **Journal of Virology**, 79(24): 15511-15524. PMC1316022

2. Gene pathways to regulate viral replication. In collaboration with researchers at the University of Wisconsin Madison and Pacific Northwest National Laboratories, I have been working to identify specific host gene networks and pathways that regulate lethal human respiratory virus replication and pathogenesis. Specifically, I was interested in determining genes that regulate SARS-CoV and MERS-CoV replication in human cell lines, models of the human conducting airway and mouse models.

a) Sims AC, Tilton SC, Menachery VD, Gralinski LE, Schäfer A, Matzke MM, Webb-Robertson BM, Chang J, Luna ML, Long CE, Shukla AK, Bankhead AR, Burkett SE, Zornetzer G, Tseng CK, Metz TO, Pickles R, McWeeney S, Smith RD, Katze MG, Waters KM, and Baric RS (2013). Release of SARS-CoV Nuclear Import Block Enhances Host Transcription in Human Lung Cells. Journal of Virology, 87(7): 3885-902. PMC3624188

b) Mitchell HD, Eisfeld AJ, Sims AC, Waters KM. A Network Integration Approach to Identify Highly Conserved Regulatory Targets Related to Pathogenicity for Influenza and SARS-CoV Respiratory Viruses. **PLoS ONE** 8(7): e69374. PMC3723910

c) Menachery VD, Eisfeld AJ, Josset L, Sims AC, Schaefer A, Proll S, Fan S, Li C, Neumann G, Tilton SC, Chang J, Gralinski LE, Long C, Green R, Matzke MM, Webb-Robertson BJ, Shukula AK, Burkett S, Metz TO, Pickles R, Smith RD, Waters KM, Katze M, Kawaoka Y, Baric RS (2014) Pathogenic influenza and coronaviruses utilize similar and contrasting approaches to control global ISG responses. mBio, 5(3). PMC4030454

d) Aevermann BD, Pickett BE, Kumar S, Sims AC, Sova P, Tam VC, Tchitchek N, Thomas PG, Tilton SC, Totura A, Wang J, Webb-Robertson B, Wen J, Weiss J, Yang J, Yount B, Zhang Q, McWeeney S, Smith RD, Waters KM, Kawaoka Y, Baric RS, Aderem A, Katze MM, Scheuermann R (2014). A Comprehensive Collection of Systems Biology Data Characterizing the Host Response to Viral Infection. **Nature's Scientific Data**,1(10). 1038/sdata.2014.33. PMC4410982

Complete List of Published Work in NCBI MyBibliography:

http://www.ncbi.nlm.nih.gov/myncbi/collections/bibliography/49189460/

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

U19-AI106772-01 (PI: Kawaoka) 06/01/13-05/31/19 Univ. of Wisconsin/NIH
MERS-CoV Supplement for OMICs Proposal
The proposed studies will provide a more detailed look at the intracellular environment by taking “snapshots” of the lipids, metabolytes, and proteins present during viral infection time courses.These assays will allow us to determine the innate immune response occurring immediately following virus infection and to determine how the virus and cell interact over a 72 hour window.
Role: Project PI

U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/19 UAB/NIH/NIAID
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.
Role: Investigator

U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/28/19 Columbia/NIH/NIAID
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.
Role: Investigator

R01 AI110700 (PI: Baric) 04/01/15-03/31/20 NIH
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.
Role: Investigator

1R01 AI132178-01 (MPI:Sheahan/Baric) 08/06/17-07/31/22 NIH
Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV
In partnership with Gilead Sciences, we aim to accelerate the preclinical development of GS-5734 and promote IND licensure. We define the pharmacokinetics, pharmacodynamics, resistance profile, efficacy breadth and mechanism of action of GS-5734 against MERS-CoV and related emerging CoV.
Role: Investigator

Completed Research Support

Contract 576652 (PI:Katze) 09/26/08-09/25/13 University of Washington/NIAID
Systems Biology of Lethal and Attenuated SARS-CoV Infection
The overall hypothesis is that highly pathogenic respiratory viruses use common and unique strategies to mechanistically remodel the intracellular environment to enhance virus replication, regulate disease severity and promote virus transmission.Using SARS-CoV and H1N1 2009 and a comparative systems biology approach with H5N1 avian influenza virus we will identify unique and common signaling circuitry that is essential for promoting severe disease profiles in the lung.
Role: Co-Investigator

Supplement to OMIC Pilot Award (PI: Kawaoka) 6/1/14-5/31/16 Univ. of Wisconsin/NIH/NIAID
Epigenetic Regulation of Interferon-Stimulated Genes Following MERS-CoV Infection
The overriding hypothesis of this supplemental application is that MERS-CoV and H5N1 manipulate host epigenetic programs to specifically down-regulate certain classes of ISGs, which likely antagonize virus replication efficiency in vitro.The goal is to develop systems biology datasets and unbiased modeling algorithms to deconvolute the complex pathogen-host interactions that regulate severe disease outcomes following infection and identify common host pathways/genes that can be exploited for therapeutic control.

Role: Project PI

U19-AI100625 (PI: Baric) 8/05/12-07/31/17 NIH/NIAID
Systems Immunogenetics of Biodefense Pathogens in the Collaborative Cross
Specific Aims:In this proposal, we are utilizing the Collaborative Cross (CC), a novel panel of reproducible, recombinant inbred (RI) mouse lines to identify genes and gene interactions, which regulate the induction, kinetics, and magnitude of the innate, inflammatory and adaptive arms of the immune response following virus infection.Specifically, we will develop novel modeling algorithms to predict and validate the causal relationships between natural genetic variation and host signaling networks, immune cell recruitment, and immune function.
Role: Investigator and Co-Education Director

Supplement to OMIC (PI: Kawaoka) 6/1/16-5/31/17 Univ. of Wisconsin/NIH/NIAID
Systems Virology for MERS-CoV in vivo
The goal is to develop systems biology datasets and unbiased modeling algorithms to deconvolute the complex pathogen-host interactions that regulate severe disease outcomes following infection and identify common host pathways/genes that can be exploited for therapeutic control.These studies will build on our current data set by collecting data sets for MERS-CoV in vivo.
Role: Project PI

[redacted] (b) (4)

The overall goal of this project is to test [redacted] (b) (4) protease inhibitor/interferon cocktails in comparison to and with nucleoside analog compounds to determine the best course of treatment for patients infected with highly pathogenic human coronaviruses.

Not Assigned (PI: Baric) 08/01/17-06/30/18 Emory/NIH
Elucidating the potential of nucleoside analog, EIDD-1931, as a broad-spectrum antiviral against highly pathogenic human coronavirus strains
To define the activity, potency and mechanism of action of EIDD-1931 against highly pathogenic human coronaviruses for development as potential therapeutic.
Role: Investigator

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Emily Ann Hagan	Research Scientist
eRA COMMONS USER NAME (b) (6)	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YYYY	FIELD OF STUDY
Hiram College	BA	05/2008	Biol. Biomed. Humanities
Columbia Univ. Mailman Sch. Public Health	MPH	05/2013	Epidemiology
Columbia Univ. Mailman Sch. Public Health	CPH	08/2013	Public Health

A. Personal Statement

I have a background in laboratory science, veterinary science, epidemiology, and human behavioral health. My main focus in the current proposed R01 work is on the human behavioral work in Aim 2. My experience in understanding the implications of laboratory testing, in conducting and analyzing quantitative and qualitative human behavioral risk assessment and mixed-methods data analysis are exactly the tools required to conduct this work. As assistant to the Senior Behavioral Risk Scientist on the USAID-EPT-PREDICT project I have regularly applied behavioral analytical skills to research data from 28 countries. I have also conducted my own focused work in Bangladesh, analyzing the results of 2 years of survey work on zoonotic viral spillover risk – directly applicable to the current proposal.

B. Positions and Honors

Positions and Employment

2006-2007	Researcher, Hiram College, Hiram College, Cellular and Molecular Lab, Hiram, OH
2007	NSF REU Research Intern, University of Akron, Polymer Department, Akron, OH
2007-2008	Teaching Assistant, Hiram College, Organic Chemistry Department, Hiram, OH
2008-2012	RA, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA
2011-2012	Team Manager, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA
2013-2015	RA, EcoHealth Alliance, New York, NY
2015-2016	Research Coordinator, EcoHealth Alliance, New York, NY
2016-2018	Research Scientist and PREDICT Bangladesh Country Liaison, EcoHealth Alliance, NY

Other Experience and Professional Memberships

2013-	*EcoHealth*, reviewer
2016-	WHO Bulletin, reviewer
2018-	*PLOS Neglected Tropical Diseases*, reviewer

Honors

2008	Biology Departmental Honors, Hiram College

C. Contribution to Science

1. **Human behavioral risk analysis.** I have helped design, coordinate training for, and conduct on-the-ground human behavioral work for the USAID-EPT-PREDICT project. This work focuses on the risk of zoonotic spillover of novel viruses from wildlife to people at high-risk interfaces in developing countries, and is therefore directly applicable to the research proposed for this R01. I have conducted field survey work in

China and Bangladesh and have published two papers directly from this work. I am currently drafting 4 manuscripts concerning behavioral risk discoveries in multiple countries.

a) Wang N, Li S, Yang X, Huang H, Zhang Y, Guo H, Luo C, Miller M, Zhu G, Chmura AA, Hagan E, Zhou J, Zhang Y, Wang L, Daszak P, Shi Z (2018). Serological evidence of bat SARS-related coronavirus infection in humans, China. **Virologica Sinica**, 33(1), 104-107.

b) Miller M, Hagan E (2017). Integrated biological–behavioural surveillance in pandemic-threat warning systems. **Bulletin of the World Health Organization**, 95(1), 62.

2. **Research collaboration in developing countries.** I have worked to provide support in analyzing and reporting novel serological, quantitative, and qualitative findings from in-country staff in developing countries. This has taken the form of training local anthropologists, physicians, clinicians, and social scientists in ethical data collection, methods of quantitative and qualitative data analysis, usage of the R statistical software, usage of the MAXQDA qualitative analysis software, and understanding the norms of scientific journal article preparation and submission. My scientific training and interdisciplinary professional experiences will be useful in the current proposed work through coordinating survey and sample collection work in China.

a) Miller M, Hagan E (2017). Integrated biological–behavioural surveillance in pandemic-threat warning systems. **Bulletin of the World Health Organization**, 95(1), 62.

b) Wang N, Li S, Yang X, Huang H, Zhang Y, Guo H, Luo C, Miller M, Zhu G, Chmura AA, Hagan E, Zhou J, Zhang Y, Wang L, Daszak P, Shi Z (2018). Serological evidence of bat SARS-related coronavirus infection in humans, China. **Virologica Sinica**, 33(1), 104-107.

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01 AI110964 Daszak (PI) 06/01/14-05/31/19
NIAID: Understanding the Risk of Bat Coronavirus Emergence
Bat ecological, human risk behavioral and virological studies to understand the risk of bat coronavirus emergence
Role: Research Scientist

USAID EPT PREDICT-2 Mazet (PI) 10/01/14 – 09/30/19
Conducting surveillance for novel pathogens in wildlife, livestock and people; characterizing human risk behavior; modeling risk of novel disease emergence; identifying mitigation strategies
The goal of this project is to assist focal countries in monitoring viruses with pandemic potential, as well as the behaviors, practices, and conditions that are associated with viral evolution, spillover, amplification, and spread.
Role: Research Scientist

Completed Research Support

USAID EPT PREDICT-1 Mazet (PI) 10/01/09 – 09/30/14
Modeling hotspots for disease emergence and conducting surveillance in wildlife in hotspots for new emerging zoonoses

This project preceded PREDICT-2, described above. $75 million award to identify hotspots of emerging diseases of pandemic potential and to help guide surveillance activities and disease control and prevention strategies across several countries.
Role: Research Scientist

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Guangjian Zhu	Co-Investigator
eRA COMMONS USER NAME [redacted] (b) (6)	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
East China Normal University, Shanghai, China	BS	07/03	Biology Science
Hainan Normal University, Haikou, China	MS	07/03	Ecology
East China Normal University, Shanghai, China	PHD	6/12	Biochemistry/Mol. Biol.

A. Personal Statement

Throughout my graduate studies and work with East China Normal University, I have carried out molecular biology and field ecological research focused on bat genetics and viral diversity. I have co-authored multiple publications in the field of viral genetics and bat ecology under the mentorship of Drs. Daszak (EcoHealth Alliance) and Shi (Wuhan Inst. Virol.). For the past 9 years I have been the in-country coordinator for all EcoHealth Alliance work in China on USAID- EPT-PREDICT, as well as for the previous NIAID R01 on bat CoVs. During this time I have been responsible for the identification of high-risk interfaces between wildlife and people, where close contact might allow for zoonotic pathogen spillover. I have also led wildlife surveys which involved bat and rodent capture and sampling for viral discovery. Through this work I have led site-selection and wild and domestic animal sampling in Guangxi, Yunnan, Guangdong and Shanghai, and have compiled archived and current samples from birds in Shanghai Chongming Reserve for H7N9 avian influenza analyses. Under the USAID PREDICT program I collected over 1,000 bat samples which have been tested for coronaviruses and other viral families at the Wuhan Institute of Virology. Under our previous NIAID award (R01AI110964), I am responsible for developing and leading a wildlife team to sample bats, rodents, and other small mammals in the live animal markets of southern China. I will continue these efforts in our renewal proposal as we focus our efforts on centers of CoV diversity in southern China. Through my graduate and professional work I have developed expertise in collecting high-quality, non-destructive samples from wildlife as well as expertise in molecular diagnostics.

B. Positions and Honors

Positions and Employment

2007- Assistant Researcher, Guangdong Entomological Institute, China

Other Experience and Professional Memberships

Honors

2009 Biology Prize of the 2009 Ig Nobel Prize (Tan *et al.* 2009, PLoS One)

C. Selected peer-reviewed publications most relevant to the current application

Ge XY, Li JL, Yang X-L, Chmura AA, Zhu G, Epstein JH, Mazet JK, Hu B, Zhang W, Peng C, Zhang YJ, Luo CM, Tan B, Wang N, Zhu Y, Crameri G, Zhang SY, Wang LF, Daszak P, Shi Z (2013). Isolation and characterization of a bat SARS-like Coronavirus that uses the ACE2 receptor. **Nature** 503: 535-538.

Zhu G, Han N, Hong T, Tan M, Yu D, Zhang L (2008). Echolocation Call, Roost and ND 1 Sequence Analysis of New Record of *Nyctalus plancyi* (Chiroptera: Vespertilionidae) on Hainan Island. **Zoological Research**, 29(4): 447-451.

Zhu G, Li D, Ye J, Hong T, Zhang L (2008). New Record of *Ia io* in Hainan Island, its Echolocation Pulses and ND1 Analysis. **Chinese Journal of Zoology**, 43(5): 69-75.

Sun Y, Yu D, Zhu G, Liu X, Zhang SY, Chen J (2009). Isolation and characterization of 11 microsatellite loci in *Scotophilus kuhlii* (Lesser Asiatic Yellow House Bat). **Conservation Genetics**, 10: 1857-1859.

Mao X, Zhu G, Zhang SY, Rossiter SJ (2010). Pleistocene climatic cycling drives intra-specific diversification in the intermediate horseshoe bat (*Rhinolophus affinis*) in Southern China. **Molecular Ecology**, 19(13): 2754-2769.

Hua P, Zhang L, Zhu G, Jones G, Zhang SY, Rossiter SJ (2011). Hierarchical polygyny in multiparous lesser flat-headed bats. **Molecular Ecology**, 20(17): 3669-3680.

Additional recent publications of importance to the field (in chronological oder)

Mazet JAK, Wei Q, Zhao G, Cummings DAT, Desmond JS, Rosenthal J, King CH, Cao W, Chmura AA, Hagan EA, Zhang S, Xiao X, Xu J, Shi Z, Feng F, Liu X, Pan W, Zhu G, Zuo G, Daszak P (2015). Joint China-Us Call for Employing a Transdisciplinary Approach to Emerging Infectious Diseases. **EcoHealth** 12(4): 555-559.

Hu B, Chmura AA, Li J, Zhu G, Desmond JS, Zhang YJ, Zhang JS, Epstein JH, Daszak P, Shi Z (2014). Detection of Diverse Novel Astroviruses from Small Mammals in China. **Journal of General Virology** 95: 2442-2449.

Zhu G, Wang R, Xuan F, Daszak P, Anthony SJ, Zhang SY, Zhang L, He G (2013). Characterization of Recombinant H9n2 Influenza Viruses Isolated from Wild Ducks in China. **Veterinary Microbiology** 166(4): 327-336.

Zhu G, Chmura AA, Zhang L (2011). Morphology, echolocation calls and diet of *Scotophilus kuhlii* (Chiroptera: Vespertilionidae) on Hainan Island, south China. **Acta Chiropterologica**, 14(1): 175-181**.**

Ma J, Jones G, Zhu G, Metzner W (2010). Echolocation behaviours of the Japanese pipistrelle bat *Pipistrellus abramus* during foraging flight. **Acta Theriologica**, 55(4): 315-332.

Tan M, Jones G, Zhu G, Ye J, Hong T, Zhou S, Zhang S, Zhang L (2009). Fellatio by fruit bats prolongs copulation time. **PLOS One**, 4(10), e7595.

Zhang L, Zhu G, Jones G, Zhang SY (2009). Conservation of bats in China: problems and recommendations. **ORYX**, 43(2): 179-182.

Zhu G, Tang Z, Liang B, Zhang X (2007). Diet and Roost Site of *Cynopterus sphinx* in Winter in Haikou. **Chinese Journal of Zoology**, 42(4): 22-27.

D. Research Support

Ongoing Research Support

USAID EPT PREDICT-1 Mazet (PI) 10/01/09 – 09/30/14

Modeling hotspots for disease emergence and conducting surveillance in wildlife in hotspots for new emerging zoonoses
Amount: $18 million subcontract on a $75 million award
Role: Lead Field Scientist

1R01AI110964 Daszak (PI) 06/01/14 – 05/31/19
NIAID: Understanding the Risk of Bat Coronavirus Emergence
Bat ecological, human risk behavioral and virological studies to understand the risk of bat coronavirus emergence
Amount: $2.5 million
Role: Lead Field Scientist

Completed Research Support

USAID EPT PREDICT-1 Mazet (PI) 10/01/09 – 09/30/14
Modeling hotspots for disease emergence and conducting surveillance in wildlife in hotspots for new emerging zoonoses
Amount: $18 million subcontract on a $75 million award
Role: Lead Field Scientist

1 R01AI079231 Daszak (PI) 09/18/08 – 08/31/13
NIAID Non-Biodefense Emerging Infectious Diseases
Risk of viral emergence from bats.
To model hotspots for bat viral diversity, identify & characterize new bat viruses & understand their pathology
Role: Research Scientist

BIOGRAPHICAL SKETCH

NAME Linfa Wang	POSITION TITLE Co-Investigator
eRA COMMONS USER NAME (b) (6)	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
East China Normal University, Shanghai	BS	01/1982	Biology
University of California, Davis	PHD	07/2086	Biochemistry

A. PERSONAL STATEMENT

My 20+ years of research focused on designing and applying novel testing platforms to discover zoonotic pathogens has direct applicability to the current proposal. I am trained as a biochemist and molecular biologist, and have been working in the field of virology and infectious diseases for more than 20 years, playing a key role in identification of animal links with several high profile zoonotic agents, including Hendra virus in Australia, Nipah virus in Malaysia and SARS virus in China. During this time, I've directed largescale laboratory diagnostic studies based on serological and PCR surveys of wildlife, domestic animals and people; and worked with multidisciplinary lab, field and modeling teams, including those at EcoHealth Alliance, to interpret the results. In my current role as director of the Program in Emerging Infectious Diseases at Duke-NUS Graduate Medical School, I have initiated major projects to continue this work, and to analyze bat genomics and basic bat biology to better understand bat-unique biological features such as longevity and co-existence with pathogens with no or minimal clinical disease. This work has led to a number of patented techniques, as well as novel reagents that I have made available to collaborators and the greater scientific community. Over the years, I have established an extensive collaborative network with scientists all around the world, covering research and surveillance work into infections of human, animal and wildlife in a truly One Health approach, including many of the collaborators on the current proposal.

Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, Wang H, Crameri G, Hu Z, Zhang H, Zhang J, McEachern J, Field H, Daszak P, Eaton BT, Zhang S, Wang LF (2005) Bats are natural reservoir of SARS-like coronaviruses. **Science** 310: 676-679.

Ge XY, Li JL, Yang X-L, Chmura AA, Zhu G, Epstein JH, Mazet JK, Hu B, Zhang W, Peng C, Zhang YJ, Luo CM, Tan B, Wang N, Zhu Y, Crameri G, Zhang SY, Wang LF, Daszak P, Shi Z (2013). Isolation and characterization of a bat SARS-like Coronavirus that uses the ACE2 receptor. **Nature** 503: 535-538.

Zhang G, Cowled C, Shi Z, Huang Z, Bishop-Lilly KA, Fang X, Wynne JW, Xiong Z, Baker ML, Zhao W, Tachedjian M, Zhu Y, Zhou P, Jiang X, Ng J, Yang L, Wu L, Xiao J, Feng Y, Chen Y, Sun X, Zhang Y, Marsh GA, Crameri G, Broder CC, Frey KG, Wang LF Wang J (2013) Comparative Analysis of Bat Genomes Provides Insight into the Evolution of Flight and Immunity. **Science** 339: 456-60.

Zhou P, Fan H, Lan T, Yang XL, Shi WF, Zhang W, Zhu Y, Zhang YW, Xie QM, Mani S, Zheng XS, Li B, Li JM, Guo H, Pei GQ, An XP, Chen JW, Zhou L, Mai KJ, Wu ZX, Li D, Anderson DE, Zhang LB, Li SY, Mi ZQ, He TT, Cong F, Guo PJ, Huang R, Luo Y, Liu XL, Chen J, Huang Y, Sun Q, Zhang XL, Wang YY, Xing SZ, Chen YS, Sun Y, Li J, Daszak P, Wang LF, Shi ZL, Tong YG, Ma JY (2018). Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. **Nature** 556: 255–258

Patents: A protease deficient Bacillus subtilis mutant strain US patent No. 5,585,253; Bacillus subtilis expression and secretion system. US patent No. 7,238,560; Footrot antigens, vaccines and diagnostic assays.

Australian Patent No. 38377/93; A novel epitope tagging system for protein surveillance and purification.
Australian Patent No. PM7419/94; Assay for the Parallel Detection of Biological Material Based on PCR.
PCT/SG2013/000455; A Chimeric Animal Comprising Stably Transplanted Bat Cells. IMC/P/10031/00/SG

B. POSITIONS AND HONORS

Positions and Employment

1986 - 89	Post-doctoral Fellow, Department of Biochemistry, University of California, Davis, USA
1990	Senior Research Officer, Centre for Molecular Biology and Medicine, Monash University, Australia
1990 - 92	Research Scientist, CSIRO Australian Animal Health Laboratory (AAHL), Geelong, Australia
1992 - 96	Senior Research Scientist, CSIRO AAHL, Geelong, Australia
1996 - 04	Principal Research Scientist, CSIRO AAHL, Geelong, Australia
2004 - 08	Senior Principal Research Scientist, CSIRO AAHL, Geelong, Australia
2008 - 15	OCE Science Leader, CSIRO AAHL, Geelong, Australia
2012 -	Professor & Director, Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore

Other Experience and Professional Memberships

1996 -	Editorial Board, *Asia Pacific J. Mol. Biol. Biotech.*
2003	WHO SARS Scientific Research Advisory Committee
2005 -	Honorary Professor, Wuhan Institute of Virology, Chinese Academy of Sciences
2006 -	Editorial Board, *Chinese J. Virol.*; *Zoonoses & Publ. Hlth.*
2006 - 7	NH & MRC Grant Review Panel
2008 -	Chair, ICTV Study Group, Paramyxoviridae
2009 -	Honorary Professor, University of Melbourne, Australia
2010 -	Editorial Board, *Frontiers Virol.*
2012 -	Editor-in-Chief, *Virol. J.*
2012 -	Board of Directors, Singapore Eye Research Institute
2012 -	Executive Committee, Australasian Society of Virology
2013 -	WHO International Health Regulations, Roster of Experts
2017 -	World Economic Forum, Global Health Threat Advisory Board

Selected Awards/Honors:

2006	CSIRO Award for Excellence in Partnership
2007	Finalist, Eureka Prize for Scientific Research
2008	CSIRO CEO Science Leader Award
2010	Elected fellow of the Australian Academy of Technological Sciences and Engineering
2011	Gardner Lecture Award, European Society of Clinical Virologist
2013	CSIRO Chairman's Medal for Research
2014	Winner, Eureka Prize for Infectious Disease Research
2014	Finalist, Prime Minister's Science Award, Australia

C. CONTRIBUTION TO SCIENCE

1. **Application of both molecular and serological platforms to pathogen discovery**

   My work at CSIRO AAHL, and now at Duke-NUS has focused on the development and use of PCR and serological assays to identify novel pathogens in wildlife, livestock and people, often under outbreak conditions. This includes the discovery of bats as a reservoir for SARS-CoV, using novel serological assays and PCR techniques I developed.

a. Bossart KN, McEacherna JA, Hickey AC, Choudhry V, Dimitrov DS, Eaton BT, Wang LF (2007) Neutralization assays for differential henipavirus serology using Bio-Plex Protein Array Systems. **Journal of Virological Methods,** 142: 29-40.

b. Thalmann CM, Cummins DM, Yu M, Lunt R, Pritchard LI, Hansson E, Crameri S, Hyatt A, Wang LF (2010) Broome virus, a new fusogenic Orthoreovirus species isolated from an Australian fruit bat. **Virology** 402:26-40.

c. Cui J, Tachedjian G, Tachedjian M, Holmes EC, Zhang SY, Wang LF (2012) Identification of diverse groups of endogenous gammaretroviruses in mega- and microbats. **Journal of General Virology** 93:2037-2045.

d. Wang J, Selleck P, Yu M, Ha W, Rootes C, Gales R, Wise T, Crameri S, Chen H, Broz I, Hyatt A, Woods R, Meehan B, McCullough S, Wang LF (2014) Novel Phlebovirus with Zoonotic Potential Isolated from Ticks, Australia. **Emerging Infectious Diseases** 20:1040-1043.

2. **Identification of bats as major reservoir of emerging zoonotic viruses**

I have used surveillance in wildlife, livestock and humans, coupled with experimental infections under BSL-2, -3, and -4, and laboratory assays to identify evidence that bats are the reservoir for a series of emerging viruses in people, including Hendra virus, Nipah virus, SARS-CoV, and others. This work has been one of the foundations for current interest in bats in emerging infectious disease research.

a. Eaton BT, Broder CC, Middleton D, and Wang LF, (2006). Hendra and Nipah viruses: different and dangerous. **Nature Reviews Microbiology**, 4: 23-35.

b. Chua KB, Crameri C, Hyatt A, Yu M, Tompang MR, Rosli J, McEachern J, Crameri S, Kumarasamy V, Eaton BT, Wang LF (2007). A previously unknown reovirus of bat origin is associated with an acute respiratory disease in humans. **Proceedings of the National Academy of Sciences**, 27: 11424-11429.

c. Mahalingam S, Herrero LJ, Playford G, Spann K, Herring B, Rolph R, Middleton D, McCall B, Field H, Wang LF (2012) Hendra virus: an emerging paramyxovirus in Australia. **Lancet Infectious Diseases** 12: 799-807.

d. Clayton BA, Middleton D, Arkinstall R, Frazer L, Wang LF, Marsh GA (2016) The Nature of Exposure Drives Transmission of Nipah Viruses from Malaysia and Bangladesh in Ferrets. **PLOS Neglected Tropical Diseases,** 10(6): e0004775.

3. **Establishment of bats as a new mammalian model system to study virus-host interaction and evolutionary biology**

Working with collaborators around the world, my lab has amassed an unprecedented collection of serological, tissue and other samples from bat surveillance programs. I have used these to develop and disseminate primary and immortalized bat cell lines, and a host of reagents which my team and collaborators are using to test hypotheses about why bats are able to host so many distinct viruses. Current projects include bat genomics and proteomics; examining the bat MHC, using gene knockout technology to identify links between flight, viral resistance, and longevity.

a. Wynne JW, Shiell BJ, Marsh G, Boyd V, Monaghan P, Zhou P, Klein R, Todd S, Mok L, Green D, Tachedjian M, Baker M, Matthews D, Wang LF (2014). Proteomics informed by transcriptomics reveals Hendra virus sensitizes bat cells to TRAIL mediated apoptosis. **Genome Biology** 15: 532.

b. Zhou P, Tachedjian M, Wynne JW, Boyd V, Cui J, Smith I, Cowled C, Ng JH, Mok L, Michalski WP, Mendenhall IH, Tachedjian G, Wang LF, Baker ML (2016). Contraction of the type I IFN locus and unusual constitutive expression of IFN-α in bats. **Proceedings of the National Academy of Sciences**, 113: 2696-2701.

c. Xie J, Li Y, Shen X, Goh G, Zhu Y, Cui J, Wang LF, Shi Z, Zhou P (2018). Dampened STING-Dependent Interferon Activation in Bats. **Cell Host and Microbe**, 23(3):297-301.

d. Yong KSM, Ng JHJ, Her Z, Hey YY, Tan SY, Tan WWS, Irac SE, Liu M, Chan XY, Gunawan M, Foo RJH, Low DHW, Mendenhall IH, Chionh YT, Dutertre CA, Chen Q, Wang LF (2018). Bat-mouse bone marrow chimera: a novel animal model for dissecting the uniqueness of the bat immune system. **Science Reports**, 8(1):4726.

D. RESEARCH SUPPORT

Ongoing research support

NRF2012NRF-CRP001-056 Wang (PI) 01/11/13-31/10/18
National Research Foundation (NRF, Singapore)
Learning from bats: from genomics to controlling viral infection and combating cancer
Using bats as model to study immunology, inflammation and other cellular/molecular mechanisms which are responsible for the unique biological features of bats, such as longevity and infection with no or less diseases.
Role: Leading PI

AI212961 Crump (PI) 01/02/16-31/01/21
NIH
Investigating Febrile Deaths in Tanzania (INDITe)
To identify actionable patient management and health system interventions that could avert fatal outcomes among patients with severe febrile illness in low-resource areas.
Role: Co-PI

[redacted] (b) (4)

Development of multiple serological platforms for differentiation of Zika and dengue virus infections

Using multiple multiplex serological platforms to develop antibody tests which can differentiation infections of Zika virus from Dengue virus and other closely related flaviviruses.
Role: PI

Completed

[redacted] (b) (4)

Establishment of serological diagnostic capability for highly virulent zoonotic viral infections in Singapore

Using most advanced technological platforms to enhance the capability in diagnosing and responding to future zoonotic disease outbreaks in Singapore.
Role: PI

[redacted] (b) (4)

Understanding the host pathogen relationships of Hendra Virus in bats, horses and humans
Examines why bats can be infected with Hendra Virus with no apparent symptoms, yet the virus causes severe disease in other mammals including humans. We hope this information can be used to design new drugs or vaccines to Hendra Virus.
Role: Co-PI

[redacted] (b) (4)

Improving the management of an emerging viral disease In Australia: determination of the mechanisms of neuroinvasion by Hendra Virus and their control, leading to optimization of post-exposure therapy following contact with Hendra Virus
Using a recently established mouse infection model, this study aims to elucidate the mechanism of Hendra Virus neuroinvasion and to optimize the post-exposure therapy strategies.
Role: Chief Investigaor

[redacted] (b) (4)

New targets in antiviral therapies
Development of novel antiviral strategies based on the interruption of nuclear localization process of key virus proteins in the families of *Paramyxoviridae* and *Rhabdoviridae*.
Role: Co-PI

AI077995 Broder (PI) 01/06/07-31/05/13
NIH/NIAID
Vaccines and therapeutics for Nipah and Hendra Virus
Establish virus infection, lethal dose, and detection parameters of Nipah virus in a ferret model. 2. Evaluate the protective efficacy of recombinant sG as a subunit vaccine for Nipah virus in the ferret. 3. Determine the passive protective efficacy of neutralizing, anti-G, fully-human monoclonal antibody therapy for Nipah virus infection in the cat and ferret. 4. Determine the solution structure of Nipah sG and in complex with its receptor ephrinB2.
Role: Co-PI

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Lili Ren	Co-Investigator
eRA COMMONS USER NAME (credential, e.g., agency login) (b) (6)	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
Norman Bethune Univ. Med. Sci., China	BS	07/1998	Clinical medicine
Medical College, Jilin university	MS	07/2002	Pathology, pathophysiol.
Ctr. Disease Control & Prevention, China	Ph.D	07/2005	Immunology

A. Personal Statement

I have expertise in the pathogenesis and evolution of respiratory viruses. Using systems biological concepts and technical systems, my group has identified several new emerging respiratory virus epidemics in China, clarifying new causative viral agents of severe pneumonia, providing insights into the pathogenic mechanisms of viruses, and improving clinical pathogen diagnostics. My group has also conducted a longitudinal study to analyze evolution of known common respiratory viruses since 2005 – the largest and the longest continuous study of its type in China. My multiple pathogen detection system approach has been transferred to more than 300 national sentinel sites. In addition, my group has established several platforms to identify the serological epidemiology of common respiratory viruses including human CoVs. This will be a critical reference for the proposed R01 work, and have implications on the design of our serological screening platform. I have also established a collaborative group with clinicians in China to study the ecology of emerging viral pathogens. My extensive experience in this type of study will greatly assist in my role overseeing clinical sample collection, and screening by PCR and serological methods in southern China.

B. Positions and Honors.

Positions and Employment

2005-2010 Research assistant, Institute of Pathogen Biology, Chinese Academy of Medical Sciences/Peking Union Medical College, China

2010-2016, Research scientist, Institute of Pathogen Biology, Chinese Academy of Medical Sciences/Peking Union Medical College, China

2016- Senior Scientist, Institute of Pathogen Biology, Chinese Academy of Medical Sciences/Peking Union Medical College, China

Other Experience and Professional Memberships

2016-2019 Member, Expert Committee on Biosafety Assessment of the National Health and Family Planning Commission

2014-2020 Member, Youth Committee of the Chinese Medical Association Medical Virology Branch

2016-2021 Member, China Research Hospital, Space Microbiology and Infection Committee Branch

2017- Editorial board, *Chinese Journal of Experimental & Clinical Virology*

Honors

2008 - 10	Outstanding Young Talents of New Century (NCET-07-0506), granted by Ministry of Education, China. Principal Investigator.
2015	2nd prize of the advanced science and technology progress award, second author, granted by Ministry of Education, China.
2016	1st prize of the advanced science and technology progress award, second author, granted by Ministry of Education, China.
2017	Excellent teacher of Peking Union Medical College (PUMC), China

C. Selected peer-reviewed publications most relevant to the current application

* = Co-corresponding or first authors

Ren L*, Richard G*, Wang Z*, Xiang Z*, Wang Y*, Zhou H, Li J, Xiao Y, Yang Q, Zhang J, Chen L, Wang W, Li Y, Li T, Meng X, Zhang Y, Guy V, Chen J, Jin Q, Wang J (2009) Prevalence of human respiratory viruses in adults with acute respiratory tract infections in Beijing, 2005–2007. **Clinical Microbiology and Infection**,15(12): 1146-1153.

Ren L, Gonzalez R, Xu X, Li J, Zhang J, Vernet G, Paranhos-Baccalà G, Jin Q, Wang J (2009) WU polyomavirus in fecal specimens of children with acute gastroenteritis, China. **Emerging Infectious Diseases**, 15(1): 134-135.

Ren L, Gonzalez R, Xiao Y, Xu X, Chen L, Vernet G, Paranhos-Baccalà G, Jin Q, Wang J (2009) Saffold cardiovirus in children with acute gastroenteritis, Beijing, China. **Emerging Infectious Diseases** 15(9): 1509-1511.

Ren L, Gonzalez R, Xu J, Xiao Y, Li Y, Zhou H, Li J, Yang Q, Zhang J, Chen L, Wang W, Vernet G, Paranhos-Baccalà G, Wang Z, Wang J (2011). Prevalence of human coronaviruses in adults with acute respiratory tract infections in Beijing, China. **Journal of Medical Virology** 83(2): 291-297.

Yang J*, Yang F*, Ren L*, Xiong Z, Wu Z, Dong J, Sun L, Zhang T, Hu Y, Du J, Wang J, Jin Q (2011). Unbiased parallel detection of viral pathogens in clinical samples by use of a metagenomic approach. **Journal of Clinical Microbiology**, 49(10): 3463-3469.

Guo L*, Zhang X*, Ren L*, Yu X*, Chen L*, Zhou H, Gao X, Teng Z, Li J, Hu J, Wu C, Xiao X, Zhu Y, Wang Q, Pang X, Jin Q, Wu F, Wang J (2014). Human antibody responses to avian influenza A(H7N9) virus, 2013. **Emerging Infectious Diseases**, 20(2): 192-200.

Ren L*, Yu X*, Zhao B*, Wu F, Jin Q, Zhang X, Wang J (2014). Infection with possible precursor of avian influenza A(H7N9) virus in a Child, China, 2013, **Emerging Infectious Diseases**, 20(8): 1362-1365.

Ren L*, Zhang Y*, Li J, Xiao Y, Zhang J, Wang Y, Chen L, Paranhos-Baccalà G, Wang J (2015). Genetic drift of human coronavirus OC43 spike gene during adaptive evolution. **Scientific Reports**, 5:11451. doi.org/10.1038/srep11451

Zhang Y, Li J, Xiao Y, Zhang J, Wang Y, Chen L, Paranhos-Baccalà G, Ren L*, Wang J* (2015). Genotype shift in human coronavirus OC43 and emergence of a novel genotype by natural recombination. **Journal of Infection**, 70(6): 641-650.

Yan F, Xiao Y, Li M, Zhang H, Zhang R, Zhou H, Shen H, Wang J, Li W*, Ren L* (2017). Metagenomic analysis identified human rhinovirus B91 infection in an adult suffering from severe pneumonia. **American Journal of Respiratory and Critical Care Medicine**, 195(11):1535-1536.

Ren L*, Yang D*, Ren X*, Li M, Mu X, Wang Q, Cao J, Hu K, Yan C, Fan H, Li X, Chen Y, Wang R, An F, An S, Luo M, Wang Y, Xiao Y, Xiang Z, Xiao Y, Li L, Huang F, Jin Q, Gao Z, Wang J (2017). Genotyping of human rhinovirus in adult patients with acute respiratory virus infections identified predominant infections of genotype A21. **Scientific Reports**, 7:41601.

Ren L*, Zhang R*, Rao J, Xiao Y, Zhang Z, Yang B, Cao D, Zhong H, Ning P, Shang H, Li M, Gao Z, Wang J (2018). Transcriptionally Active Lung Microbiome and Its Association with Bacterial Biomass and Host Inflammatory Status. **mSystems**, 3:e00199-18.

D. Research Support

Ongoing Research Support

1. 2017ZX10103004, Key project of infectious diseases 01/01/2017-12/31/2020
Viral etiology and spectrum of respiratory tract infections and the mutations characteristics
The goal of this project is to identify the etiology of community acquired pneumonia in China and the epidemic and mutations of the important respiratory viruses.
Role: PI

Completed Research Support

1. 2012ZX10004-206 Key project of infectious diseases 01/01/2012-12/31/2015
Viral etiology and spectrum of respiratory tract infections and the mutations characteristics
The goal of this project is to investigate the etiology of acute respiratory tract infections in China and the sero-epidemiological of important respiratory viruses.
Role: PI

2. 2009ZX10004-206 Key project of infectious diseases 01/01/2009-12/31/2010
Viral etiology and spectrum of respiratory tract infections and the mutations characteristics
The goal of this project is to establish the surveillance network and to investigate the etiology of acute respiratory tract infections in China.
Role: PI

BIOGRAPHICAL SKETCH

NAME Li Guo	POSITION TITLE Co-Investigator
eRA COMMONS USER NAME (b) (6)	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
Bengbu Medical College, China	B.S.	07/1997	Clinical Medicine
Anhui Medical University, China	M.S.	07/2002	Microbiology
Chinese Center for Disease Control & Prevention	M.D.	07/2006	Immunology

A. Personal Statement

I have direct expertise in the proposed work described in this R01. I have been working on the etiology and immunology of respiratory viruses since 2003. I have evaluated cross-reactivity of NP among HCoVs and developed a competitive ELISA (cELISA) for detecting anti-N IgG antibodies against HCoV -229E, -OC43, -NL63, and -HKU1. These data indicate differential transmission of HCoVs in the Chinese population and that anti-N IgG may serves as an index for susceptibility to HCoV infections. In addition, I have evaluated human antibody responses to A(H7N9) influenza virus, differential seroprevalence of human bocavirus species 1-4 in China, and found bocavirus in children with respiratory tract infections.

B. Positions and Honors.

Positions and Employment

1997 - 99	Teaching Assistant, Bengbu Medical College
2002 - 09	Assistant Professor, Chinese Center for Disease Control and Prevention
2009 - 15	Associate Professor, Institute of Pathogen Biology (IPB), Chinese Academy of Medical Science (CAMS) /Peking Union Medical College.
2015 -	Professor, Institute of Pathogen Biology (IPB), Chinese Academy of Medical Science (CAMS) /Peking Union Medical College

C. Selected peer-reviewed publications

* = Co-corresponding or first author

Guo L, Wang D, Zhou H, Wu C, Gao X, Xiao Y, Ren L, Paranhos-Baccalà G, Shu Y, Jin Q, Wang J (2016). Cross-reactivity between avian influenza A (H7N9) virus and divergent H7 subtypic and heterosubtypic influenza A viruses. **Scientific Reports**, 6, 22045.

Gao X, Zhou H, Wu C, Xiao Y, Ren L, Paranhos-Baccalà G, Guo L*, Wang J* (2015). Antibody against nucleocapsid protein predicts susceptibility to human coronavirus infection. **Journal of Infection** 71(5): 599-602.

Chen Z*, Wang J*, Bao L*, Guo L*, Zhang W, Xue Y, Zhou H, Xiao Y, Wang J, Wu F, Deng Y, Qin C, Jin Q (2015). Human monoclonal antibodies targeting the haemagglutinin glycoprotein can neutralize H7N9 influenza virus. **Nature Communications**, 6:6714.

Yang J*, Zhang T*, Guo L*, Hu YF, Li JL, Su HX, Xiao Y, Ren XW, Dong J, Sun LL, Xiao Y, Li Li, Yang F, Wang JW, Yuan H, Jin Q (2014). Mutations of Novel Influenza A(H10N8) Virus in Chicken Eggs and MDCK Cells. **Emergining Infectious Diseases**, 20(9):1541-1543.

Zhou Z, Gao X, Wang Y, Zhou H, Wu C, Paranhos-Baccalà G, Vernet G, Guo L***,** Wang J* (2014). Conserved B-Cell Epitopes among Human Bocavirus Species Indicate Potential Diagnostic Targets. **PLOS One**, 9(1): e86960.

Guo L, Zhang X, Ren L, Yu X, Chen L, Zhou H, Gao X, Teng Z, Li J, Hu J, Wu C, Xiao X, Zhu Y, Wang Q, Pang X, Jin Q, Wu F, Wang J (2014). Human antibody responses to avian influenza A(H7N9) virus. **Emerging Infectious Diseases**, 20(2): 192-200.

Guo L, Wu C, Zhou H, Wu C, Paranhos-Baccalà G, Vernet G, Jin Q, Wang J, Hung T (2013). Identification of a nonstructural DNA-binding protein (DBP) as an antigen with diagnostic potential for human adenovirus. **PLOS One**, 8(3): e56708.

Guo L, Wang Y, Zhou H, Wu C, Song J, Li J, Paranhos-Baccalà G, Vernet G, Wang J, Hung T (2012). Differential seroprevalence of human bocavirus species 1-4 in Beijing, China. **PLOS One**, 7(6): e39644.

Guo L, Gonzalez R, Zhou H, Wu C, Vernet G, Wang Z, Wang J (2012). Detection of three human adenovirus species in adults with acute respiratory infection in China. **European Journal of Clinical Microbiology and Infectious Disease**, 31(6): 1051-1058.

Guo L, Gonzalez R, Xie Z, Zhou H, Liu C, Wu C, Paranhos-Baccalà G, Vernet G, Shen K, Jin Q, Wang J (2011). Bocavirus in children with respiratory tract infections. **Emerging Infectious Diseases**, 17(9): 1775-1777.

Wang Y, Gonzalez R, Zhou H, Li J, Li Y, Paranhos-Baccalà G, Vernet G, Guo L*, Wang J* (2011). Detection of human bocavirus 3 in China. **European Journal of Clinical Microbiology and Infectious Disease**, 30(6): 799-805.

Guo L, Gonzalez R, Wang W, Vernet G, Paranhos-Baccalà G, Wang J (2010). Complete genome sequence of human astrovirus genotype 6. **Virology Journal**, 7: 29.

Guo L, Xu X, Song J, Wang W, Wang J, Hung T (2010). Molecular Characterization of Astrovirus Infection in Children with Diarrhea in Beijing, 2005-2007. **Journal of Medical Virology**, 82(3): 415-423.

Guo L, Zhou H, Wang M, Song J, Han B, Shu Y, Ren L, Si H, Qu J, Zhao Z, Wang J, Hung T (2009). A recombinant adenovirus prime-virus-like particle boost regimen elicits effective and specific immunities against norovirus in mice. **Vaccine**, 27(38): 5233-5238.

Guo L, Song J, Xu X, Ren L, Li J, Zhou H, Wang M, Qu J, Wang J, Hung T (2009). Genetic analysis of norovirus in children affected by acute gastroenteritis in Beijing, 2004-2007. **Journal of Clinical Virology** 44(1): 94-98.

Guo L, Wang J, Zhou H, Si H, Wang M, Song J, Han B, Shu Y, Ren L, Qu J, Hung T (2008) Intranasal administration of a recombinant adenovirus expressing the norovirus capsid protein stimulates specific humoral, mucosal, and cellular immune responses in mice. **Vaccine**, 26(4): 460-468.

D. Research Support

Ongoing Research Support

2018ZX10734404-006 01/01/2018-12/31/2020

Key technologies for the identification and identification of important respiratory viruses and establishment of reference libraries
Role: Co-PI

Completed Research Support

(b) (4) (b) (4)

Study on the immunoprotection of recombinant adenovirus vaccine against Norovirus by using virus-like particles as a control
Role: PI

BIOGRAPHICAL SKETCH

NAME Peng Zhou	POSITION TITLE Co-Investigator
eRA COMMONS USER NAME (b) (6)	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
College of Life Science, Henan University, China	BS	07/04	Bioengineering
Wuhan Inst. Virol., Chinese Acad. Sci., China	PHD	01/11	Virology
Australian Animal Health Laboratory, CSIRO	Postdoc	07/14	Viral Immunology
Singapore Duke-NUS Medical School, Singapore	Postdoc	02/16	Viral Immunology

A. Personal Statement

My virological expertise is directly related to the proposed work in this R01 renewal, including next generation diagnostic tool development for monitoring bat virus spillover, bat pathogen discovery and bat viral immunology. I have worked on bat virology since 2004, and participated in the work that led to the discovery of SARS-like coronaviruses in Mainland China. I worked on bat viral immunology to explore reasons why bats can coexist with high viral diversity and viral loads. My findings show that in bats, a constitutively expressed interferon and a dampened STING-dependent interferon production pathway exists, which may explain why bats can control viral replication and tolerate viral diseases. My most recent work on Swine Acute Diarrhea Syndrome (SADS) characterized the spillover of CoV from bats to swine causing a large-scale pandemic. I have worked closely with Dr. Linfa Wang from Singapore Duke-NUS medical school, on developing next-generation viral nucleotide, serological and isolation tools for coronavirus from bats and other animals. I have also collaborated with, and published papers with Dr. Daszak and other staff at EcoHealth Alliance. I will be in charge of diagnostics, genomics, and virus isolation in this project.

B. Positions and Honors.

Positions and Employment

2016- Principle Investigator, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China

Other Experience and Professional Memberships

2009 - 10 Joint PhD study in Australia Animal Health Laboratory, CSIRO

Honors

2009 Scholarship for China-Australia Joint PhD study, China Scholarship Council.
2017 Natural Science Award (the first rank) of Hubei province, China.
2018 National Science Fund for Excellent Young Scholars, China.

C. Selected peer-reviewed most relevant to the current application

* = Co-corresponding or first authors

1. Zhou P, Fan H, Lan T, Yang XL, Shi WF, Zhang W, Zhu Y, Zhang YW, Xie QM, Mani S, Zheng XS, Li B, Li JM, Guo H, Pei GQ, An XP, Chen JW, Zhou L, Mai KJ, Wu ZX, Li D, Anderson D, Zhang LB, Li SY, Mi ZQ, He TT, Cong F, Guo PJ, Huang R, Luo Y, Liu XL, Chen J, Huang Y, Sun Q, Zhang XLL, Wang YY, Xing SZ, Chen YS, Sun Y, Li J, Daszak P, Wang LF, Shi ZL, Tong YG, Ma JY (2018) Fatal swine acute diarrhea syndrome

caused by an HKU-2 related coronavirus of bat origin. **Nature**, 556: 255-258. doi.org/10.1038/s41586-018-0010-9

2. Xie J, Li Y, She X, Goh G, Zhu Y, Cui J, Wang LF, Shi Z, Zhou P (2018) Dampened STING-dependent interferon activation in bats. **Cell Host and Microbes** 23(3): 297-301. doi.org/10.1016/j.chom.2018.01.006
3. Zhou P, Tachedjian M, Wynne JW, Boyd V, Cui J, Smith I, Cowled C, Ng JH, Mok L, Michalski WP, Mendenhall IH, Tachedjian G, Wang LF, Baker ML (2016). Contraction of the type I IFN locus and unusual constitutive expression of IFN-α in bats. **Proceedings of the National Academy of Sciences**, 113(10): 2696-701. doi.org/10.1073/pnas.1518240113
4. Wu L*, Zhou P*, Ge XY, Wang LF, Baker ML, Shi Z (2013). Deep RNA Sequencing Reveals Complex Transcriptional Landscape of a Bat Adenovirus. **Journal of Virology**, 87(1): 503-511. doi.org/10.1128/JVI.02332-12
5. Zhou P, Li H, Wang H, Wang LF, Shi Z (2012). Bat severe acute respiratory syndrome-like coronavirus ORF3b homologues display different interferon antagonist activities. **Journal of General Virology**, 93: 275-281. doi.org/10.1099/vir.0.033589-0
6. Zhou P, Cowled C, Todd S, Crameri G, Virtue ER, Marsh GA, Shi ZL, Wang LF, and Baker ML (2011). Type III Interferons in pteropid bats: differential expression patterns provide evidence for distinct roles in antiviral immunity. **Journal of Immunology**,186(5): 3138-3147. doi.org/ 10.4049/jimmunol.1003115
7. Zhou P, Han Z, Wang LF, Shi Z (2009). Immunogenicity difference between the SARS coronavirus and the bat SARS-like coronavirus spike (S) proteins. **Biochemal Biophysical Research Communications**, 387(2): 326-329. doi.org/10.1016/j.bbrc.2009.07.025

D. Research Support

Ongoing Research Support

[redacted] (b) (4)

Combating the next SARS- or MERS-like emerging infectious disease outbreak by improving active surveillance
Role: PI

[redacted] (b) (4)

Interferon responses in SARS-Like Coronavirus infected Bat cells
Role: PI

[redacted] (b) (4)

Bat virology
Role: PI

Completed Research Support

[redacted] (b) (4)

Immune responses and transcriptome analysis of bat adenovirus infected bat cells
Role: Co-PI

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Ben Hu	Co-Investigator
eRA COMMONS USER NAME (b) (6)	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
Huazhong Agricultural University, China	BS	2007	Veterinary medicine
Huazhong Agricultural University, China	MS	2010	Preventive vet. med.
Wuhan Inst. Virology, Chinese Acad. Sci.	PHD	2015	Microbiology

A. Personal Statement

I obtained my PhD degree in 2015 and have been working as a Research Assistant in Dr. Zhengli Shi's laboratory for over 3 years. My research is focused on the discovery and characterization of viruses in small mammals, especially in bats and rodents. My work forms the basis for some of the key findings built on in the current R01 proposal, in particular: 1) the identification of diverse bat SARSr-CoVs at cave sites in Yunnan, China; 2) the potential recombination origin of SARS-CoV; and 3) characterization of spillover risk for bat SARSr-CoVs. I have also reported genetically diverse novel astroviruses in bats, rodents, and shrews in China. I have collaborated with EcoHealth Alliance scientists on multiple research projects for the past 3 years.

B. Positions and Honors.

Positions and Employment

2015- Assistant Researcher, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China

Honors

C. Selected peer-reviewed publications most relevant to the current application

Note: * = Corresponding Author

1. Ge X, Li J, Yang X, Chmura AA, Zhu G, Epstein JH, Mazet JK, Hu B, Zhang W, Peng C, Zhang YJ, Luo CM, Tan B, Wang N, Zhu Y, Crameri G, Zhang SY, Wang LF, Daszak P*, Shi Z (2013). Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. **Nature**, 503: 535-538. doi.org/10.1038/nature12711

2. Hu B, Chmura AA, Li J, Zhu G, Desmond JS, Zhang Y, Zhang W, Epstein JH, Daszak P, Shi Z (2014). Detection of diverse novel astroviruses from small mammals in China. **Journal of General Virology** 95, 2442-2449. doi.org/10.1099/vir.0.067686-0

3. Hu B, Ge X, Wang LF, Shi Z (2015). Bat origin of human coronaviruses. **Virology Journal**, 12(1): 221. doi.org/10.1186/s12985-015-0422-1

4. Ge XY, Wang N, Zhang W, Hu B, Li B, Zhang YZ, Zhou JH, Luo CM, Yang XL, Wu LJ, Wang B, Zhang Y, Li ZX, Shi Z (2016). Coexistence of multiple coronaviruses in several bat colonies in an abandoned mineshaft. **Virologica Sinica**, 31(1): 31-40. doi.org/10.1007/s12250-016-3713-9

5. Wang MN, Zhang W, Gao YT, Hu B, Ge XY, Yang XL, Zhang YZ, Shi Z (2016). Longitudinal surveillance of SARS-like coronaviruses in bats by quantitative real-time PCR. **Virologica Sinica**, 31(1): 78-80. doi.org/ 10.1007/s12250-015-3703-3

6. Yang XL, Hu B, Wang B, Wang MN, Zhang Q, Zhang W, Wu LJ, Ge XY, Zhang YZ, Daszak P, Wang LF, Shi Z (2016). Isolation and Characterization of a Novel Bat Coronavirus Closely Related to the Direct Progenitor of Severe Acute Respiratory Syndrome Coronavirus. **Journal of Virology**, 90(6): 3253-3256. doi.org/ 10.1128/JVI.02582-15

7. Waruhiu C, Ommeh S, Obanda V, Agwanda B, Gakuya F, Ge XY, Yang XL, Wu LJ, Zohaib A, Hu B, Shi Z (2017). Molecular detection of viruses in Kenyan bats and discovery of novel astroviruses, caliciviruses, and rotaviruses. **Virologica Sinica**, 32(2):101-114. doi.org/ 10.1007/s12250-016-3930-2

8. Hu B, Zeng LP, Yang XL, Ge XY, Zhang W, Li B, Xie JZ, Shen XR, Zhang YZ, Wang N, Luo DS, Zheng XS, Wang MN, Daszak P, Wang LF, Cui J, Shi Z (2017). Discovery of A Rich Gene Pool of Bat SARS-related Coronaviruses Provides New Insights into the Origin of SARS Coronavirus. **PLOS Pathogens**, 13(11): e1006698.

9. Luo CM, Wang N, Yang XL, Liu HZ, Zhang W, Li B, Hu B, Peng C, Geng QB, Zhu GJ, Li F, Shi Z (2018). Discovery of Novel Bat Coronaviruses in South China That Use the Same Receptor as Middle East Respiratory Syndrome Coronavirus. **Journal of Virology**, 92(13): e00116-18.

10. Luo Y, Li B, Jiang RD, Hu BJ, Luo DS, Zhu GJ, Hu B, Liu HZ, Zhang YZ, Yang XL, Shi ZL (2018). Longitudinal Surveillance of Betacoronaviruses in Fruit Bats in Yunnan Province, China during 2009-2016. **Virologica Sinica**, 33(1):87-95. doi.org/ 10.1007/s12250-018-0017-2

D. Research Support
Ongoing Research Support

31800142 Hu (PI) 01/01/2019-12/31/2021
National Natural Science Foundation of China
Pathogenicity studies of two novel bat SARSr-CoVs on transgenic mice expressing human ACE2
Role: PI

R01 AI110964 Daszak (PI) 06/01/14-05/31/19
Understanding Risk of Bat Coronaviruses
The goal of this study is to analyze the risk of coronavirus spillover from bats to humans in Southern China
Role: Research Scientist

Emerging Pandemic Threat Program, USAID Mazet (PI) 10/01/14-09/30/19
PREDICT 2
The goal of this project is to create and implement a global virus surveillance system in animals and humans and analyze spillover risk.
Role: Laboratory Scientist

Completed Research Support
USAID EPT PREDICT-1 Mazet (PI) 10/01/09 – 09/30/14
Modeling hotspots for disease emergence and conducting surveillance in wildlife in hotspots for new emerging zoonoses
Amount: $18 million subcontract on a $75 million award
Role: Laboratory Scientist

BIOGRAPHICAL SKETCH

NAME Aleksei A. Chmura	POSITION TITLE Research Scientist
eRA COMMONS USER NAME (b) (6)	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
Columbia University	BS	06/2004	Biology
School of Life Sciences, Kingston University (UK)	PhD	08/2018	Biology

A. Personal Statement

Dr. Chmura has an interdisciplinary background in ecology, wildlife biology, virology, and extensive on-the-ground experience conducting wildlife sampling in China. Fluent in spoken and written Mandarin, for the past decade, Dr. Chmura has acted as a key coordinator among EcoHealth Alliance headquarters staff, and laboratory and field teams in China. Dr. Chmura's personal research involves the wildlife origins of SARS-CoV, wildlife paramyxovirus diversity and evolution, and human-wildlife contact behavior in southern China. His work has been funded by USAID EPT/PREDICT since 2009. As part of his doctoral work, he spent over a year in the Wuhan Institute of Virology laboratory in China under the direction of Dr. Zhengli Shi and Dr. Peter Daszak.

B. Positions and Honors.

Positions and Employment

2001-2004, Volunteer Curator, Dept. of Mammalogy, American Museum of Natural History, USA
2001-2005, Program Assistant, Ctr. Environmental Research and Conservation, Columbia University, USA
2002-2005, Instructor, Columbia University Tropical Field Ecology Programs, USA/Domician Republic/Brazil
2005-Present, Program Coordinator, EcoHealth Alliance, USA
2006-Present, Managing Editor, *EcoHealth*, New York, USA

Other Experience and Professional Memberships

2000-2005	The Explorers Club
2002-present	American Museum of Natural History
2005-present	International Association for Ecology and Health
2009-present	Society for Applied Microbiology

C. Selected peer-reviewed publications most relevant to the current application

Monagin C, Ning L, Schneider B, Chmura AA, Epstein JH, Wu D, Paccha B, Ke C, Daszak P, Rabinowitz P (2018) Serologic and behavioral risk survey of workers with wildlife contact in China. **PLOS ONE**, 13(4): e0194647.

Wang N, Li, S, Yang X, Huang H, Zhang Y, Guo H, Luo C, Miller M, Zhu G, Chmura AA, Hagan E, Zhou J, Zhang Y, Wang L, Daszak P, Shi Z (2018) Serological evidence of bat SARS-related coronavirus infection in humans, China. **Virologica Sinica**, 33(1): 104-107.

Zeng L, Ge X, Peng C, Yang X, Tan B, Gao Y, Chen J, Chmura AA, Daszak P, Shi Z (2016) Bat Severe Acute Respiratory Syndrome-Like Coronavirus WIV1 Encodes an Extra Accessory Protein, ORFX, Involved in Modulation of the Host Immune Response. **Journal of Virology**, 90(14): 6573–6582.

Mazet JAK, Wei Q, Zhao G, Cummings DAT, Desmond JS, Rosenthal J, King CH, Cao W, Chmura AA, Hagan EA, Zhang S, Xiao X, Xu J, Shi Z, Feng F, Liu X, Pan W, Zhu G, Zuo G, Daszak P (2015). Joint China-Us Call for Employing a Transdisciplinary Approach to Emerging Infectious Diseases. **EcoHealth** 12(4): 555-559.

Hu B, Chmura AA, Li J, Zhu G, Desmond JS, Zhang YJ, Zhang JS, Epstein JH, Daszak P, Shi Z (2014). Detection of Diverse Novel Astroviruses from Small Mammals in China. **Journal of General Virology** 95: 2442-2449.

Ge XY, Li JL, Yang X-L, Chmura AA, Zhu G, Epstein JH, Mazet JK, Hu B, Zhang W, Peng C, Zhang YJ, Luo CM, Tan B, Wang N, Zhu Y, Crameri G, Zhang SY, Wang LF, Daszak P, Shi Z (2013). Isolation and characterization of a bat SARS-like Coronavirus that uses the ACE2 receptor. **Nature** 503: 535-538.

Zhu G, Chmura AA, Zhang L (2011). Morphology, echolocation calls and diet of *Scotophilus kuhlii* (Chiroptera: Vespertilionidae) on Hainan Island, south China. **Acta Chiropterologica**, 14(1): 175-181**.**

Kilpatrick AM, Chmura AA, Gibbons DW, Fleischer RC, Marra PP, Daszak P (2006). Predicting the global spread of H5N1 avian influenza. **PNAS** 103: 19368-19373.

D. Research Support

Ongoing Research Support

R01 AI110964 Daszak (PI) 06/01/14-05/31/19
Understanding Risk of Bat Coronaviruses
The goal of this study is to analyze the risk of coronavirus spillover from bats to humans in Southern China
Role: Research Scientist

Emerging Pandemic Threat Program, USAID Mazet (PI) 10/01/14-09/30/19
PREDICT 2
The goal of this project is to create and implement a global virus surveillance system in animals and humans and analyze spillover risk.
Role: Program Coordinator

Completed Research Support
USAID EPT PREDICT-1 Mazet (PI) 10/01/09 – 09/30/14
Modeling hotspots for disease emergence and conducting surveillance in wildlife in hotspots for new emerging zoonoses
Role: Program Coordinator

2 R01TW005869 Daszak (PI) 09/01/08 – 08/31/13
NIH Ecology of Infectious Diseases (Fogarty International Center)
The Ecology, Emergence and Pandemic Potential of Nipah virus in Bangladesh
To conduct mathematical modeling and fieldwork to understand the dynamics of Nipah virus in Bangladesh
Role: Research Scientist

NSF DEB-1257513 Daszak (PI) 08/15/12-07/31/13
US-China Ecology and Evolution of Infectious Diseases Collaborative Workshop; Kunming, China 2012
Role: Program Coordinator

1 R01AI079231 Daszak (PI) 09/18/08 – 08/31/13
NIAID Non-Biodefense Emerging Infectious Diseases
Risk of viral emergence from bats.
To model hotspots for bat viral diversity, identify & characterize new bat viruses & understand their pathology
Role: Research Scientist

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 0770900660000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: ECOHEALTH ALLIANCE, INC.

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period:** 1

A. Senior/Key Person

(b) (4), (b) (6)

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	PETER		DASZAK		PD/PI		[redacted]					
2.	Dr.	Kevin	J	Olival		Co-Investigator		[redacted]					
3.	Dr.	Leilani	V	Francisco		Co-Investigator		[redacted]					
4.	Dr.	Noam		Ross		Co-Investigator		[redacted]					
5.	Ms.	Hongying		Li		Research Scientist		[redacted]					
6.	Dr.	Alice		Latinne		Research Scientist		[redacted]					
7.	Ms.	Emily	A	Hagan		Research Scientist		[redacted]					
8.	Dr.	Aleksei	A	Chmura		Research Scientist		[redacted]					

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **223,713.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**223,713.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 0770900660000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: ECOHEALTH ALLIANCE, INC.

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period:** 1

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	9,440.00
2. Foreign Travel Costs	29,958.00
Total Travel Cost	**39,398.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 0770900660000

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: ECOHEALTH ALLIANCE, INC.

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period:** 1

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	20,850.00
2. Publication Costs	
3. Consultant Services	79,750.00
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	190,649.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**291,249.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**554,360.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . EcoHealth Alliance Indirect Cost	32.74	363,710.00	119,079.00
2 . EcoHealth Alliance Indirect Cost on 3 Subawards (IPB, UNC, WIV)	32.74	75,000.00	24,555.00
3 . University of North Carolina at Chapel Hill Indirect Cost	55.5	50,000.00	27,750.00
4 . IPB and WIV Subawards (2) Indirect Costs	8.0	140,649.00	11,252.00
		Total Indirect Costs	**182,636.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**736,996.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	736,996.00

L. Budget Justification*

File Name: EHA_NIAID_COV_BUDGET_JUSTIFICATION_FINAL.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 0770900660000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: ECOHEALTH ALLIANCE, INC.

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	PETER		DASZAK		PD/PI							(b) (4), (b) (6)
2 .	Dr.	Kevin	J	Olival		Co-Investigator							
3 .	Dr.	Leilani	V	Francisco		Co-Investigator							
4 .	Dr.	Noam		Ross		Co-Investigator							
5 .	Ms.	Hongying		Li		Research Scientist							
6 .	Dr.	Alice		Latinne		Research Scientist							
7 .	Ms.	Emily	A	Hagan		Research Scientist							
8 .	Dr.	Aleksei	A	Chmura		Research Scientist							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **223,713.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**223,713.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 0770900660000

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: ECOHEALTH ALLIANCE, INC.

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period:** 2

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	9,440.00
2. Foreign Travel Costs	29,958.00
Total Travel Cost	**39,398.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 0770900660000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: ECOHEALTH ALLIANCE, INC.

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period:** 2

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	14,850.00
2. Publication Costs	6,000.00
3. Consultant Services	79,750.00
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	190,649.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**291,249.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**554,360.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . EcoHealth Alliance Indirect Cost	32.74	363,710.00	119,079.00
2 . University of North Carolina at Chapel Hill Indirect Cost	55.5	50,000.00	27,750.00
3 . IPB and WIV Subawards (2) Indirect Costs	8.0	140,649.00	11,252.00
		Total Indirect Costs	**158,081.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**712,441.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	712,441.00

L. Budget Justification*	File Name: EHA_NIAID_COV_BUDGET_JUSTIFICATION_FINAL.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 0770900660000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: ECOHEALTH ALLIANCE, INC.

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period: 3**

A. Senior/Key Person

(b) (4), (b) (6)

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	PETER		DASZAK		PD/PI		[redacted]					
2.	Dr.	Kevin	J	Olival		Co-Investigator		[redacted]					
3.	Dr.	Leilani	V	Francisco		Co-Investigator		[redacted]					
4.	Dr.	Noam		Ross		Co-Investigator		[redacted]					
5.	Ms.	Hongying		Li		Research Scientist		[redacted]					
6.	Dr.	Alice		Latinne		Research Scientist		[redacted]					
7.	Ms.	Emily	A	Hagan		Research Scientist		[redacted]					
8.	Dr.	Aleksei	A	Chmura		Research Scientist		[redacted]					

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **223,713.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**223,713.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 0770900660000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: ECOHEALTH ALLIANCE, INC.

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period:** 3

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	9,440.00
2. Foreign Travel Costs	29,958.00
Total Travel Cost	**39,398.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 0770900660000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: ECOHEALTH ALLIANCE, INC.

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period:** 3

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	14,850.00
2. Publication Costs	6,000.00
3. Consultant Services	79,750.00
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	190,649.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**291,249.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**554,360.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . EcoHealth Alliance Indirect Cost	32.74	363,710.00	119,079.00
2 . University of North Carolina at Chapel Hill Indirect Cost	55.5	50,000.00	27,750.00
3 . IPB and WIV Subawards (2) Indirect Costs	8.0	140,649.00	11,252.00
		Total Indirect Costs	**158,081.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**712,441.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	712,441.00

L. Budget Justification*	File Name: EHA_NIAID_COV_BUDGET_JUSTIFICATION_FINAL.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 0770900660000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: ECOHEALTH ALLIANCE, INC.

Start Date*: 06-01-2022 **End Date*:** 05-31-2023 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	PETER		DASZAK		PD/PI		[redacted]					(b) (4), (b) (6)
2.	Dr.	Kevin	J	Olival		Co-Investigator		[redacted]					
3.	Dr.	Leilani	V	Francisco		Co-Investigator		[redacted]					
4.	Dr.	Noam		Ross		Co-Investigator		[redacted]					
5.	Ms.	Hongying		Li		Research Scientist		[redacted]					
6.	Dr.	Alice		Latinne		Research Scientist		[redacted]					
7.	Ms.	Emily	A	Hagan		Research Scientist		[redacted]					
8.	Dr.	Aleksei	A	Chmura		Research Scientist		[redacted]					

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **223,713.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**223,713.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 0770900660000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: ECOHEALTH ALLIANCE, INC.

Start Date*: 06-01-2022 **End Date*:** 05-31-2023 **Budget Period:** 4

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	9,440.00
2. Foreign Travel Costs	29,958.00
Total Travel Cost	**39,398.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 0770900660000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: ECOHEALTH ALLIANCE, INC.

Start Date*: 06-01-2022 **End Date*:** 05-31-2023 **Budget Period:** 4

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	14,850.00
2. Publication Costs	6,000.00
3. Consultant Services	79,750.00
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	190,649.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**291,249.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**554,360.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . EcoHealth Alliance Indirect Cost	32.74	363,710.00	119,079.00
2 . University of North Carolina at Chapel Hill Indirect Cost	55.5	50,000.00	27,750.00
3 . IPB and WIV Subawards (2) Indirect Costs	8.0	140,649.00	11,252.00
		Total Indirect Costs	**158,081.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**712,441.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	712,441.00

L. Budget Justification*	File Name: EHA_NIAID_COV_BUDGET_JUSTIFICATION_FINAL.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 0770900660000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: ECOHEALTH ALLIANCE, INC.

Start Date*: 06-01-2023 **End Date*:** 05-31-2024 **Budget Period: 5**

A. Senior/Key Person

(b) (4), (b) (6)

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	PETER		DASZAK		PD/PI		[redacted]					
2.	Dr.	Kevin	J	Olival		Co-Investigator		[redacted]					
3.	Dr.	Leilani	V	Francisco		Co-Investigator		[redacted]					
4.	Dr.	Noam		Ross		Co-Investigator		[redacted]					
5.	Ms.	Hongying		Li		Research Scientist		[redacted]					
6.	Dr.	Alice		Latinne		Research Scientist		[redacted]					
7.	Ms.	Emily	A	Hagan		Research Scientist		[redacted]					
8.	Dr.	Aleksei	A	Chmura		Research Scientist		[redacted]					

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **223,713.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**223,713.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 0770900660000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: ECOHEALTH ALLIANCE, INC.

Start Date*: 06-01-2023 **End Date*:** 05-31-2024 **Budget Period:** 5

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	9,440.00
2. Foreign Travel Costs	29,958.00
Total Travel Cost	**39,398.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 0770900660000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: ECOHEALTH ALLIANCE, INC.

Start Date*: 06-01-2023 **End Date*:** 05-31-2024 **Budget Period:** 5

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	14,850.00
2. Publication Costs	6,000.00
3. Consultant Services	79,750.00
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	190,649.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**291,249.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**554,360.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . EcoHealth Alliance Indirect Cost	32.74	363,710.00	119,079.00
2 . University of North Carolina at Chapel Hill Indirect Cost	55.5	50,000.00	27,750.00
3 . IPB and WIV Subawards (2) Indirect Costs	8.0	140,649.00	11,252.00
		Total Indirect Costs	**158,081.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**712,441.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	712,441.00

L. Budget Justification* File Name:
EHA_NIAID_COV_BUDGET_JUSTIFICATION_FINAL.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

ECOHEALTH ALLIANCE BUDGET JUSTIFICATION

A. Senior/Key personnel:

The PD/PI, Dr. Peter Daszak, will commit (b) (4), (b) (6) per year in each year of this budget. He will be primarily responsible for overseeing the project, general management, communication and collaboration with subaward partners, as well as contributing to data analysis and manuscript writing.

Co-Investigator, Dr. Kevin Olival, will commit (b) (4), (b) (6) per year in each year of this budget. Dr. Olival will lead the design and implementation of the bat sampling fieldwork (Aim 1); facilitate overall project management; and train and oversee field teams. Dr. Olival will also oversee modeling and analyses under Aims 1 & 3, participate in regular conference calls, and help write manuscripts and reports.

Co-Investigator, Dr. Leilani Francisco, will commit (b) (4), (b) (6) per year in each year of this budget. Dr. Francisco will lead the implementation of the community and clinic-based surveillance (Aim 2), including adherence to study design, sampling methodology, and ethics in human subjects research; data collection instrument development; data management, cleaning, and analysis; and, findings dissemination.

Co-Investigator, Dr. Noam Ross, will commit (b) (4), (b) (6) per year in each year of this budget. Dr. Ross will lead modeling work and assist in with data analyses and manuscript writing. He will also advise on data management, statistical approaches, and computational work.

B. Other Personnel

Research Scientist, Ms. Hongying Li, will commit (b) (4), (b) (6) per year in each year of this budget. Ms. Li will coordinate the field and laboratory activities in China, maintaining the financial administration, results reporting, and data management, as well as work closely with Dr. Lili Ren at the Institute of Pathogen Biology to refine protocols, oversee field data collection, and perform data analysis for human study.

Research Scientist, Dr. Alice Latinne, will commit (b) (4), (b) (6) per year in each year of this budget. Dr. Latinne will assist in with phylogenetic and phylogeographic analyses and manuscript writing. She will also advise on data management and field activities.

Research Scientist, Dr. Aleksei Chmura, will commit (b) (4), (b) (6) per year in each year of this budget. Dr. Chmura will coordinate regular calls, reports, maintain EcoHealth Alliance and subaward budgets and both project and financial reporting, draft subcontracts, and set-up project databases, advise field activities, assist with statistical analysis, and manuscript writing.

Research Scientist, Ms. Emily Hagan, will commit (b) (4), (b) (6) per year in each year of this budget. Ms. Hagan will assist with the development of human data collection instruments, testing, and implementation; advise on data storage, data analyses, and manuscript writing. She will also provide training for field teams conducting human subjects research.

Fringe benefits for Year 1 are calculated for EcoHealth Alliance's federally approved rate of 31.5% of base salary and is included in all subsequent years.

C. Equipment

No Equipment costing more than $5,000 will be purchased

D. Travel

Domestic Travel

$9,440 is requested annually for Years 1 through 5 for the PD/PI, 3 Co-Investigators, and 1 Research Scientist to attend and present on research results at the annual American Society for Tropical Medicine and Hygiene and the American Public Health Association meetings. 2 night and 3 day travel to Washington, DC is

calculated as follows: $205 for hotels ($251 x 2 nights x 5 people x 2 trips = $5,020); $76 for meals and incidentals ($76 x 2.5 days x 5 people x 2 trips = $1,900); and $252 for round-trip train ($252 x 5 x 2 = $2,520).

International Travel
$11,998 is requested annually in Years 1 to 5. This will support round-trip flights from New York to Beijing and Wuhan for the field annual meetings for 3 Senior/Key Personnel and 1 for the PD/PI (Daszak) at $1,055 each. Five nights and six days of hotels, meals, and incidentals for 3 Senior/Key Personal and 1 PD/PI are calculated at $1,944.50 per year: hotels at $258 per night (x 5 nights and 4 personnel = $5,160) and meals and incidentals at $119 per day (x 5.5 days and 4 personnel = $2,856).

$17,960 is requested annually in Years 1 to 5 for EHA Research Scientists (Ms. Li and Ms. Hagan) who will travel to China for two field training and supervising visits per year for duration of 21 days each. Support for this request, annually, is $17,960 and is calculated as follows: 2 round trip flights = $4,400; hotel $258 x 20 nights x twice a year = $4,732; meals and incidentals at $119 per day x 20.5 days x twice a year = $3,570

E. Participant/Trainee Support Costs
There are no participant/trainee support costs.

F. Other Direct Costs
Materials & Supplies
We request $7,000 in Year 1 for sample collection materials to be shipped to China including bat catching equipment ($1,000); PPE ($2,000); and 1 liquid nitrogen dry shipper ($1,000) for Wuhan Institute of Virology in China to be used by Dr. Guanjian Zhu for field work.

In Years 2 through 5, field and human sampling will be completely underway; we request support for PPE ($2,000) and other sample collection materials ($2,000) in each of these years.

Publication Costs
We request $6,000 per year for only Years 2 to 5 for publication fees required to publish research findings in peer-reviewed journals such as *Nature*, *Public Library of Science*, and other journals

Subawards/Consortium/Contractual Costs
We are requesting consortium/contractual support for our three partners: Wuhan Institute of Virology (WIV), Institute of Pathogen Biology (IPB), and University of North Carolina (UNC). We have fully detailed these direct and indirect costs in their respective sub-award budgets.

Computers, Software, Reference Materials and Dataset Acquisition
We request support of $6,000 to permit two Research Scientists to purchase 1 laptop each (2 x $3,000 including insurance and software). We also request $1,000 per year in each year to cover software and reference materials, and an additional $1,000 per year in each year for acquisition of datasets.

Shipping
We will be shipping the materials and supplies detailed above to our subaward institutions in China (IPB and WIV). Shipping box and all taxes are estimated at $1,667 per shipment. We estimate 3 shipments of supplies and materials will be sent every year through the duration of this project.

Consultants
Dr. Linfa Wang, Co-Investigator/Consultant We request (b) (4), (b) (6) per year for each year of the project for a consultancy for Dr. Linfa Wang who will focus on PCR development, serological testing strategy and virus characterization, and will also participate in regular meetings with collaborators. Dr. Wang has more than 20 years of research experience in designing and applying novel testing platforms to discover zoonotic pathogens.

Dr. Guangjian Zhu, Co-Investigator/Consultant In total, we request $368,000 for the consultancy of Dr. Guangjian Zhu from Year 1 to Year 5 of the project including: $204,390 for field personnel, $124,750 for field travel; $33,548 for field supplies and materials, and $5,255 for other costs. Detailed expenses are calculated as the follows:

Personnel ($204,390)
Dr. Guangjian Zhu Co-Investigator/Consultant will oversee all field sampling activities in China by coordinating with local partners and stakeholders to lead the specimen collection and bats population monitoring at selected surveillance sites. Dr. Zhu is a zoologist and ecologist specializing in bats surveillance in southern and western China and has been leading EcoHealth Alliance's field surveillance work in China for more than 15 years. We request [redacted] annual stipend for Zhu, who will commit [redacted] per year to this project in years 1-3 and increase time to [redacted] per year for in years 4-5, since he will allocate more time to collaborating on peer-reviewed publications in the last two years of the project.

Research Assistant (TBD) will assist the Co-PI and Field Coordinator (Zhu) for project data management, reporting, and administration. We request [redacted] p.a. salary for this Research Assistant who will dedicate 2 months p.a. on this project from Years 1-5.

Field Assistants (2 in each province, TBD) will assist all field surveillance activities including specimen collection and data entry and management. The assistants will commit a total of 50 days per year to this project from years 1-5. We request [redacted] per year to support each assistant for the field surveillance work.

Travel ($124,750)
Inter-Province Travel. We request 1) $1,200 per year for all five years of this project to cover 3-per-year round-trip flights/trains each from Shanghai, to Yunnan, Guangdong, Guizhou, and Guangxi for Dr. Zhu to meet with collaborating institutions, train field teams, and ensure sample collection, storage, and shipments. Each round-trip flight is estimated at $400, in total $6,000 for 5 years; 2) $2,400 per year for all five years of this project to cover 2-per-year round-trip flights/trains for 2 field assists traveling to the field sites in Yunnan, Guangdong, Guizhou, and Guangxi for sampling work. Each round-trip flight is estimated at $400, in total $12,000 for 5 years.

Field Transportation. Field work will take place for 50 days per year for 5 years, the expenses of local transportation include 1) car rental at the rate of $79/car/day, with 1 car for 50 days, in total of $3,950 per year, and $19,750 for 5 years; 2) Gas and toll fee at the rate of $32/car/day, with 1 car for 50 days, in total of $1,600 per year, and $8,000 for 5 years.

Meal and Lodging. We request 1) $6,400 to cover the expense of meals for 4 field team members in the field for 50 days per year, at the rate of $32/person/day, totaling $32,000 in 5 years; 2) $9,400 for lodging expenses of 4 field team members in the field for 50 days at the rate of $47/person/night, totaling $247,000 in 5 years.

Supplies and Materials ($33,548)
Biological sampling supplies ($25,165) We request $25,165 to purchase supplies for biological sampling during the 5 years of the project, including 1) puritan calcium alginate swabs $8,800 (5,000 IND); 2) viral sample collection tubes $6,875 (15,000 IND); 3) heparinized glass hematocrit tubes $190 (~4,000IND); 4) mist nets for bats trapping $2,200 (~500IND); 5) cloth bags for bats trapping $2,400 (~1,000IND); 6) Viral Transport Media $4,700 (~7,000 mL).

Personal Protection Equipment ($4,336): We request 1) $3,440 for 3M N95 respirators (~1,600IND) for field work across Year 1-5; 2) $470 for eye protection glasses (~100 IND) for

the use in field across Year 1-5; 3) $426 for nitrile gloves (~3,000IND) for sampling work for Year 1-5.

Cold Chain Maintenance ($4,047): We request $4,047 to purchase 3 liquid nitrogen dry shippers for preserve biological samples in the field before transported an ultra-low temperature freezer. The expense is calculated at the rate of $1,349 each, with 1 purchased per year from Year 1-3, totaling $4,047.

Equipment ($0)
No equipment over $5,000 will be purchased.

Other Costs ($6,399)
We request 1) a total of $1,275 for specimen transportation or delivery from the field to partners' labs from Year 1-5, at the rate of $85/delivery with 1,000 tubes, with three times per year; and 2) a total of $3,980 for rabies and tetanus vaccination 4 field team members from Year 1-5, at the rate of $199/year/person.

H. Indirect Costs
We are requesting the EcoHealth Alliance federally approved indirect cost rate of 32.74% on all applicable direct costs. Indirect is taken only on the first $25,000 for each consortium/contractual agreement in each year. As there are 3 (Wuhan Institute of Virology, Institute of Pathogen Biology, and University of North Carolina), a total of $24,555 ($8,185 x 3) is requested as indirect costs on consortium/contractual/subaward agreements. This is not included as part of direct cost calculations and is only requested for year 1. In years 2-5 no indirect will be taken on consortium/contractual agrrement subcontracts.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		1,118,565.00
Section B, Other Personnel		0.00
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		1,118,565.00
Section C, Equipment		0.00
Section D, Travel		196,990.00
1. Domestic	47,200.00	
2. Foreign	149,790.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		1,456,245.00
1. Materials and Supplies	80,250.00	
2. Publication Costs	24,000.00	
3. Consultant Services	398,750.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	953,245.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		2,771,800.00
Section H, Indirect Costs		814,960.00
Section I, Total Direct and Indirect Costs (G + H)		3,586,760.00
Section J, Fee		0.00
Section K, Total Costs and Fee (I + J)		3,586,760.00

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Ralph	S	Baric		Co-Investigator		(b) (4), (b) (6)					(b) (4), (b) (6)
2.	Dr.	Amy		Sims		Co-Investigator							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Laboratory Technician	(b) (4), (b) (6)					(b) (4), (b) (6)
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: The University of North Carolina at Chapel Hill

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period:** 1

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: The University of North Carolina at Chapel Hill

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period:** 1

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	15,960.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**15,960.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**50,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1 . All Direct Costs	55.5	50,000.00	27,750.00
		Total Indirect Costs	**27,750.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**77,750.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	77,750.00

L. Budget Justification* File Name: NIAID_COV_2019_UNC_BUDGET_JUSTIFICATION.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period:** 2

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Ralph	S	Baric		Co-Investigator							(b) (4), (b) (6)
2.	Dr.	Amy		Sims		Co-Investigator							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Laboratory Technician	(b) (4), (b) (6)					(b) (4), (b) (6)
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 6081952770000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: The University of North Carolina at Chapel Hill

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period:** 2

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Funding Opportunity Number: PA-18-484 . Received Date: 2018-11-05T16:31:22.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 6081952770000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: The University of North Carolina at Chapel Hill

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period:** 2

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	15,960.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**15,960.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**50,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . All Direct Costs	55.5	50,000.00	27,750.00
		Total Indirect Costs	**27,750.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**77,750.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	77,750.00

L. Budget Justification*
File Name:
NIAID_COV_2019_UNC_BUDGET_JUSTIFICATION.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Ralph	S	Baric		Co-Investigator		[redacted]					(b) (4), (b) (6)
2 .	Dr.	Amy		Sims		Co-Investigator							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** [redacted] (b) (4), (b) (6)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Laboratory Technician	[redacted] (b) (4), (b) (6)			[redacted]		(b) (4), (b) (6)
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted] (b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: The University of North Carolina at Chapel Hill

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period:** 3

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 6081952770000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: The University of North Carolina at Chapel Hill

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period:** 3

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	15,960.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**15,960.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**50,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . All Direct Costs	55.5	50,000.00	27,750.00
		Total Indirect Costs	**27,750.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**77,750.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	77,750.00

L. Budget Justification*
File Name:
NIAID_COV_2019_UNC_BUDGET_JUSTIFICATION.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 06-01-2022 **End Date*:** 05-31-2023 **Budget Period:** 4

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Ralph	S	Baric		Co-Investigator							(b) (4), (b) (6)
2.	Dr.	Amy		Sims		Co-Investigator							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Laboratory Technician	(b) (4), (b) (6)					(b) (4), (b) (6)
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: The University of North Carolina at Chapel Hill

Start Date*: 06-01-2022 **End Date*:** 05-31-2023 **Budget Period:** 4

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Funding Opportunity Number: PA-18-484 . Received Date: 2018-11-05T16:31:22.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: The University of North Carolina at Chapel Hill

Start Date*: 06-01-2022 **End Date*:** 05-31-2023 **Budget Period:** 4

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	15,960.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**15,960.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**50,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . All Direct Costs	55.5	50,000.00	27,750.00
		Total Indirect Costs	**27,750.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**77,750.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	77,750.00

L. Budget Justification* File Name: NIAID_COV_2019_UNC_BUDGET_JUSTIFICATION.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 06-01-2023 **End Date*:** 05-31-2024 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Ralph	S	Baric		Co-Investigator		[redacted]					(b) (4), (b) (6)
2 .	Dr.	Amy		Sims		Co-Investigator							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **20,319.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Laboratory Technician	(b) (4), (b) (6) [redacted]				[redacted]	(b) (4), (b) (6)
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6) [redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: The University of North Carolina at Chapel Hill

Start Date*: 06-01-2023 **End Date*:** 05-31-2024 **Budget Period:** 5

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: The University of North Carolina at Chapel Hill

Start Date*: 06-01-2023 **End Date*:** 05-31-2024 **Budget Period:** 5

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	15,960.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**15,960.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**50,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . All Direct Costs	55.5	50,000.00	27,750.00
		Total Indirect Costs	**27,750.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**77,750.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	77,750.00

L. Budget Justification* File Name:
NIAID_COV_2019_UNC_BUDGET_JUSTIFICATION.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL BUDGET JUSTIFICATION, SUBAWARD

A. Senior/Key Personnel

Ralph Baric, PhD Co-Investigator [redacted] (b) (4), (b) (6). Dr. Baric is a known expert in coronavirus cross species transmission and pathogenesis and has studied this group of viruses for over 30 years. His group developed the first reverse genetic systems for epidemic and zoonotic SARS-like coronaviruses and they have studied the ability of these viruses to replicate efficiently in various primary human airway epithelial cell cultures as well as other key primary cell types. His group has also studied the sensitivity of these viruses to be controlled by existing vaccines and therapeutics both in vitro and in vivo. Dr. Baric will lead the studies at the University of North Carolina at Chapel Hill. He will design research strategies, interpret findings and review research outcomes with Dr. Sims and Mr. Yount. At a regular basis, Dr. Baric will report the results of the teams research to Dr. Daszak and Dr. Shi and together, they will use this information to identify additional research priorities and design downstream studies. Drs. Daszak, Shi and Baric have published together in the past and participated on research project applications. Dr. Baric recently spent several days in Wuhan, China, where he discussed research strategies and collaborations with Dr. Daszak and Dr. Shi. He will work closely with Dr. Sims and Mr. Yount to prepare timely reports, share research and discuss future research directions with the group.

Amy Sims, PhD Co-Investigator [redacted] (b) (4), (b) (6). Dr. Sims has over 22 years of research studying coronavirus molecular biology, replication and pathogenesis. She has published over 50 papers including seminal papers on characterizing host response patterns of primary human lung airway epithelial cells and other cell types after infection with SARS-CoV, MERS-CoV, influenza and various SARS-like bat coronaviruses. She is not only well versed in the preparation, cultivation and maintenance of primary human lung cells but also proficient at studying virus infection outcomes, in the presence and absence of antiviral therapeutics. In consultation with Dr. Baric, Dr. Sims will design experiments, perform infections and characterize epidemic and bat SARS-like coronavirus replication in human cells. She will compile data and share these results with the research team. Dr. Sims will also interface and work closely with Mr. Yount, who will assist in these studies, including infections, cell preparations and characterizing virus growth efficiency in these cultures. Dr. Sims has over 15 years of experience working in a BSL3 laboratory and oversees the management of these facilities. She has select agent clearance.

B. Other Personnel

Mr. Boyd Yount, Laboratory Technician [redacted] (b) (4), (b) (6) Mr. Yount has published over 50 papers on coronaviruses and developed the first reverse genetic platforms for SARS-CoV, MERS-CoV and various SARS-like bat coronaviruses. He will work closely with Drs. Barics, Shi and Daszak to design and recover select bat SARS-like coronaviruses for downstream studies in the Baric and Shi laboratories, including characterizing virus phenotypes in primary cells as well as cells expressing various human, civet and bat ACE2 receptors. He will prepare virus stocks, Mr. Yount will work closely with Drs. Baric and Sims to design and implement experiments in the BSL3 laboratory, prepare reports and research outcomes during the course of the program. Mr. Yount has over 15 years of experience in a BSL3 setting and is well versed in all the techniques used in this proposal. He has select agent clearance.

Fringe Benefits. Benefits are for faculty, staff and postdoctoral research associates are calculated as follows:
Faculty and Staff – 24.519% Social Security and retirement and $6,104 for health insurance,
Supplies and Reagents. $15,960

C. Equipment

No equipment over $5,000 will be purchased.

D. Travel

No travel will be requested for this subaward. Travel to EcoHealth Alliance and other collaborators will be covered from other UNC funding.

F. Other Direct Costs
Materials and Supplies. A variety of culture media and serum ($3,000), primary cell procurement ($2500), recombinant enzymes ($1500) and antibodies ($1500), synthetic DNAs ($2,500) and an assortment of miscellaneous supplies (e.g., gloves, chemicals, plasticware, etc.)($2460) are needed during the course of the program to recover recombinant viruses and maintain cells in culture, perform virus growth curves and identify virus tropisms by immunohistochemistry. In addition, personnel protective equipment (PPE), portal breathing apparati (PAPR), globes and protective clothing are used in the BSL3 setting ($2500).

H. Indirect Costs
In an agreement with DHHS dated 11/23/2016 the indirect cost rate for The University of North Carolina is 55.5% of MTDC, excluding equipment and tuition.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		104,095.00
Section B, Other Personnel		66,105.00
Total Number Other Personnel	5	
Total Salary, Wages and Fringe Benefits (A+B)		170,200.00
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		79,800.00
1. Materials and Supplies	79,800.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		250,000.00
Section H, Indirect Costs		138,750.00
Section I, Total Direct and Indirect Costs (G + H)		388,750.00
Section J, Fee		0.00
Section K, Total Costs and Fee (I + J)		388,750.00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 5290274740000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Zhengli		Shi		Co-Investigator	[redacted]						(b) (4), (b) (6)
2.	Dr.	Peng		Zhou		Co-Investigator	[redacted]						
3.	Dr.	Ben		Hu		Co-Investigator	[redacted]						

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **17,667.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**17,667.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 5290274740000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Wuhan Institute of Virology

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period:** 1

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	4,314.00
Total Travel Cost	**4,314.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 5290274740000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Wuhan Institute of Virology

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period:** 1

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	48,668.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**48,668.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**70,649.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Direct Costs	8.0	70,648.00	5,652.00
		Total Indirect Costs	**5,652.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**76,301.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	76,301.00

L. Budget Justification* File Name:
NIAID_COV_2019_WIV_BUDGET_JUSTIFICATION.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 5290274740000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period:** 2

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Zhengli		Shi		Co-Investigator							(b) (4), (b) (6)
2 .	Dr.	Peng		Zhou		Co-Investigator							
3 .	Dr.	Ben		Hu		Co-Investigator							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **17,667.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**17,667.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 5290274740000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Wuhan Institute of Virology

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period:** 2

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	4,314.00
Total Travel Cost	**4,314.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 5290274740000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Wuhan Institute of Virology

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period:** 2

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	48,668.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**48,668.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**70,649.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Direct Costs	8.0	70,648.00	5,652.00
		Total Indirect Costs	**5,652.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**76,301.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	76,301.00

L. Budget Justification* File Name:
NIAID_COV_2019_WIV_BUDGET_JUSTIFICATION.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 5290274740000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Zhengli		Shi		Co-Investigator		[redacted]					(b) (4), (b) (6)
2 .	Dr.	Peng		Zhou		Co-Investigator							
3 .	Dr.	Ben		Hu		Co-Investigator							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **17,667.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**17,667.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 5290274740000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Wuhan Institute of Virology

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period:** 3

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	4,314.00
Total Travel Cost	**4,314.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 5290274740000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Wuhan Institute of Virology

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period:** 3

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	48,668.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**48,668.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**70,649.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Direct Costs	8.0	70,648.00	5,652.00
		Total Indirect Costs	**5,652.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**76,301.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	76,301.00

L. Budget Justification* File Name:
NIAID_COV_2019_WIV_BUDGET_JUSTIFICATION.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 5290274740000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

Start Date*: 06-01-2022 **End Date*:** 05-31-2023 **Budget Period:** 4

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Zhengli		Shi		Co-Investigator		[redacted]					(b) (4), (b) (6)
2.	Dr.	Peng		Zhou		Co-Investigator							
3.	Dr.	Ben		Hu		Co-Investigator							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **17,667.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**17,667.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 5290274740000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Wuhan Institute of Virology

Start Date*: 06-01-2022 **End Date*:** 05-31-2023 **Budget Period:** 4

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	4,314.00
Total Travel Cost	**4,314.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 5290274740000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Wuhan Institute of Virology

Start Date*: 06-01-2022 **End Date*:** 05-31-2023 **Budget Period:** 4

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	48,668.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**48,668.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**70,649.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1 . Direct Costs	8.0	70,648.00	5,652.00
		Total Indirect Costs	**5,652.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**76,301.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	76,301.00

L. Budget Justification* File Name: NIAID_COV_2019_WIV_BUDGET_JUSTIFICATION.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 5290274740000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Wuhan Institute of Virology

Start Date*: 06-01-2023 **End Date*:** 05-31-2024 **Budget Period:** 5

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Zhengli		Shi		Co-Investigator		[redacted]					(b) (4), (b) (6)
2 .	Dr.	Peng		Zhou		Co-Investigator							
3 .	Dr.	Ben		Hu		Co-Investigator							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **17,667.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**17,667.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-18-484 . Received Date: 2018-11-05T16:31:22.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 5290274740000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Wuhan Institute of Virology

Start Date*: 06-01-2023 **End Date*:** 05-31-2024 **Budget Period:** 5

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	4,314.00
Total Travel Cost	**4,314.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 5290274740000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Wuhan Institute of Virology

Start Date*: 06-01-2023 **End Date*:** 05-31-2024 **Budget Period:** 5

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	48,668.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**48,668.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**70,649.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Direct Costs	8.0	70,648.00	5,652.00
		Total Indirect Costs	**5,652.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**76,301.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	76,301.00

L. Budget Justification* File Name: NIAID_COV_2019_WIV_BUDGET_JUSTIFICATION.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

WUHAN INSTITUTE OF VIROLOGY BUDGET JUSTIFICATION, SUBAWARD

A. Senior/Key Personnel:

Dr. Zhengli Shi, Co-Investigator. Senior Research Scientist at Wuhan Institute of Virology (WIV) Chinese Academy of Sciences, will commit (b) (4), (b) (6) per year (b) (4), (b) (6) to this project to oversee the laboratory implementation at WIV. At a regular basis, Dr. Shi will meet with other Co-PIs to refine study protocols, report back results, and prepare publications. Dr. Shi has been working on the discovery and characterization of novel viruses from bats and other wildlife since 2004. This included the discovery that Chinese horseshoe bats are the natural reservoir of SARSr-CoVs and the likely origin of SARS-CoV. Her lab at WIV isolated SARSr-CoVs from bats sharing high homology with human SARS-CoV and demonstrated their interspecies transmission risk, largely confirming bats as the source of SARs. She will lead her team to carry out then systematic studies on the epidemiology, genetic evolution, interspecies infection mechanism and pathogenesis of a series of bat-borne CoVs on this R01 renewal proposal.

Dr. Peng Zhou, Co-Investigator. Research Scientist at Wuhan Institute of Virology (WIV) Chinese Academy of Sciences, will commit will commit (b) (4), (b) (6) per year (b) (4), (b) (6) to this project to be in charge of the diagnostics, genomics, and virus isolation work at WIV. Dr. Zhou have been working on bat virology since 2004, who will contribute his expertise in next generation diagnostic tool development for monitoring bat virus spillover, bat pathogen discovery, and bat viral immunology to this R01 renewal proposal.

Dr. Ben Hu, Co-Investigator. Research Associate and coordinator of Shi's laboratory at WIV, will commit (b) (4), (b) (6) (b) (4), (b) (6) per year (b) (4), (b) (6) to this project to directly supervisor the lab technicians, and coordinate with our collaborators in this R01 proposal for communication, reporting, and or organizing meetings. Dr. Hu has been working on discovery and characterization of viruses in small mammals in bats and rodents since 2010 at WIV, and had extensive experience managing international collaborative projects.

B. Equipment

No equipment over $5,000 will be purchased.

C. Travel

We are requesting $4,314 per year for all years for Dr. Shi to travel to the United States to meet with EcoHealth Alliance (Daszak, Francesco, Olival, Ross) and University of North Carolina at Chapel Hill (Baric, Sims) collaborators. Travel is calculated at one round trip airfare from Wuhan to New York City ($1,000), nine-night hotel in New York City ($288 per night), and 10 days per diem at $76 per day except for first and last day, which have a reduced per diem of $57.

D. Other Direct Costs

We are requesting support for laboratory experiments and related testing costs with a minimum base of 2,000 samples from 1,000 animals per year.

RNA Extractions

We will be running RNA Extractions for 1,000 bats per year (two samples per bat: rectal and blood) in each year of the project. This will cost $6,214 per year (QIAamp ViralRNA Mini Kit with Axygen Pipette Tips and Filter Tubes at $3.11 per sample).

RT-PCR

Costs for 1-Step RT-PCR assays for Coronavirus conducted on 2,000 samples per year for each year of the project total $6,358 and are detailed as follows: Superscript III one step kit ($2.31 per sample); Platinum Tag DNA Polymerase ($0.25 per sample); nuclease-free water ($0.07 per sample); and Axygen Pipette Tips and Filter Tubes ($0.54 per sample).

DNA Sequencing

In each year of the project, DNA Sequencing will be performed on 1,500 samples at a cost of $4.34 per reaction. We request a total of $6,503 per year in each year.

In vitro Infection Experiment
We are requesting support for *in vitro* infection experiments using pseudoviruses carrying the spike proteins (wild type or mutants) or live viruses in cell lines of different origins, binding affinity assays between the spike proteins (wild type or mutants) and different cellular receptor molecules, and humanized mouse experiments. In each year of the project, we request $1,040 for Lipofectamine3000 transfection reagent; $3,612 for GIBCO Fetal Bovine Serum, $517 for GIBCO antibiotic antimycotic, and 2,601 for GIBCO medium that will be used in the *in vitro* infection experiments that will be used for *in vitro* infection experiment, as well as $6,000 for cell lines, in total of $8,639 per year.

Luciferase Immunoprecipitation System (LIPS) Assay
We are requesting $18,642 to support the in each year of the project to develop LIPS assay for bat CoV antibody detection, with detailed cost as follows: $9,827 for Protein A/G UltraLink Resin; $434 for Monoclonal ANTI-FLAG(R) M2 antibody; $5,636 for Renilla Luciferase Assay System; $2,168 for Merck-Millipore MSBVN1B50 MultiScreen HTS; $578 for Axygen Polypropylene PCR Tube Strips.

The Enzyme-Linked Immunosorbent Assay (ELISA)
We also request $2,312 to support the serological testing of 1,000 bat serum samples per year with ELISA plates, at the cost of $2.31 per sample.

E. Indirect Costs
We are requesting an extremely low indirect cost of 8% on all direct costs.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		88,335.00
Section B, Other Personnel		0.00
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		88,335.00
Section C, Equipment		0.00
Section D, Travel		21,570.00
1. Domestic	0.00	
2. Foreign	21,570.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		243,340.00
1. Materials and Supplies	243,340.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		353,245.00
Section H, Indirect Costs		28,260.00
Section I, Total Direct and Indirect Costs (G + H)		381,505.00
Section J, Fee		0.00
Section K, Total Costs and Fee (I + J)		381,505.00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 5281563570000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Institute of Pathogen Biology

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Lili		Ren		Co-Investigator							(b) (4), (b) (6)
2.	Dr.	Li		Guo		Co-Investigator							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	(b) (4), (b) (6)

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-18-484 . Received Date: 2018-11-05T16:31:22.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 5281563570000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Institute of Pathogen Biology

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period:** 1

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	4,314.00
Total Travel Cost	**4,314.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 5281563570000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Institute of Pathogen Biology

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period:** 1

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	48,686.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**48,686.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**70,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1 . Direct Costs	8.0	70,000.00	5,600.00
		Total Indirect Costs	**5,600.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**75,600.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	75,600.00

L. Budget Justification* File Name:
NIAID_COV_2019_IPB_BUDGET_JUSTIFICATION.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 5281563570000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Institute of Pathogen Biology

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Lili		Ren		Co-Investigator							(b) (4), (b) (6)
2 .	Dr.	Li		Guo		Co-Investigator							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	(b) (4), (b) (6)

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 5281563570000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Institute of Pathogen Biology

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period:** 2

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	4,314.00
Total Travel Cost	**4,314.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 5281563570000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Institute of Pathogen Biology

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period:** 2

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	48,686.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**48,686.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**70,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Direct Costs	8.0	70,000.00	5,600.00
		Total Indirect Costs	**5,600.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**75,600.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	75,600.00

L. Budget Justification* File Name:
NIAID_COV_2019_IPB_BUDGET_JUSTIFICATION.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 5281563570000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Institute of Pathogen Biology

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period:** 3

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr.	Lili		Ren		Co-Investigator							(b) (4), (b) (6)
2.	Dr.	Li		Guo		Co-Investigator							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**

Total Salary, Wages and Fringe Benefits (A+B) (b) (4), (b) (6)

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 5281563570000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Institute of Pathogen Biology

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period:** 3

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	4,314.00
Total Travel Cost	**4,314.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 5281563570000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Institute of Pathogen Biology

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period:** 3

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	48,686.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**48,686.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**70,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Direct Costs	8.0	70,000.00	5,600.00
		Total Indirect Costs	**5,600.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**75,600.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	75,600.00

L. Budget Justification*	File Name: NIAID_COV_2019_IPB_BUDGET_JUSTIFICATION.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 5281563570000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Institute of Pathogen Biology

Start Date*: 06-01-2022 **End Date*:** 05-31-2023 **Budget Period:** 4

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Lili		Ren		Co-Investigator							(b) (4), (b) (6)
2 .	Dr.	Li		Guo		Co-Investigator							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	(b) (4), (b) (6)

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 5281563570000

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Institute of Pathogen Biology

Start Date*: 06-01-2022 **End Date*:** 05-31-2023 **Budget Period:** 4

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	4,314.00
Total Travel Cost	**4,314.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 5281563570000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Institute of Pathogen Biology

Start Date*: 06-01-2022 **End Date*:** 05-31-2023 **Budget Period:** 4

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	48,686.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**48,686.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**70,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Direct Costs	8.0	70,000.00	5,600.00
		Total Indirect Costs	**5,600.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**75,600.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	75,600.00

L. Budget Justification*	File Name: NIAID_COV_2019_IPB_BUDGET_JUSTIFICATION.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 5281563570000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Institute of Pathogen Biology

Start Date*: 06-01-2023 **End Date*:** 05-31-2024 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Lili		Ren		Co-Investigator		[redacted]					(b) (4), (b) (6)
2 .	Dr.	Li		Guo		Co-Investigator		[redacted]					

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** [redacted] (b) (4), (b) (6)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**

Total Salary, Wages and Fringe Benefits (A+B) [redacted] (b) (4), (b) (6)

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 5281563570000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Institute of Pathogen Biology

Start Date*: 06-01-2023 **End Date*:** 05-31-2024 **Budget Period:** 5

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	0.00

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	4,314.00
Total Travel Cost	**4,314.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 5281563570000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Institute of Pathogen Biology

Start Date*: 06-01-2023 **End Date*:** 05-31-2024 **Budget Period:** 5

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	48,686.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**48,686.00**

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	**70,000.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1 . Direct Costs	8.0	70,000.00	5,600.00
		Total Indirect Costs	**5,600.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	**75,600.00**

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	75,600.00

L. Budget Justification* File Name:
NIAID_COV_2019_IPB_BUDGET_JUSTIFICATION.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

WUHAN INSTITUTE OF VIROLOGY BUDGET JUSTIFICATION, SUBAWARD

A. Senior/Key Personnel:
Dr. Lili Ren, PhD Co-Investigator [redacted] (b) (4), (b) (6). Dr. Ren is an expert in the pathogenesis and evolution of respiratory viruses. She will refine study protocols, coordinate research, oversee implementation of all activities, analyze data, lead regular meetings with other Co-Investigators and Other Senior/Key Personnel as well as draft papers.

Dr. Li Guo, PhD Co-Investigator and Senior Research Technician [redacted] (b) (4), (b) (6) per year to perform all laboratory work. She has been working on etiology and immunology research on respiratory viruses since 2003 and has evaluated the cross-reactivities of N among HCoVs and developed a competitive ELISA (cELISA) for detecting anti-N IgG antibodies against HCoV -229E, -OC43, -NL63, and -HKU1.

B. Other Personnel
No other Personnel will be required for this subaward. All Institute of Pathogen Biology salaries include the US benefits, so benefits are not calculated separately.

C. Equipment
No equipment over $5,000 will be purchased.

D. Travel
We are requesting $4,314 per year for all years for Dr. Ren or Dr. Guo to travel to the United States to meet with EcoHealth Alliance (Daszak, Francesco, Olival, Ross) and University of North Carolina at Chapel Hill (Baric, Sims) collaborators. Travel is calculated at one round trip airfare from Beijing to New York City ($1,000), nine-night hotel in New York City ($288 per night), and 10 days per diem at $76 per day except for first and last day, which have a reduced per diem of $57.

F. Other Direct Costs
We are requesting support for laboratory experiments and related costs with a minimum base of 1,000 samples expected per year.

RNA Extractions
We will be running 1,000 RNA Extractions per year in each year of the project. This will cost $10,450 per year for QIAamp ViralRNA Mini Kit with an additional $2,023 per year for Axygen Pipette Tips and Filter Tubes at $1.08 per sample.

1-STEP RT-PCR
Costs for 1-Step RT-PCR assays for Coronavirus conducted on 1,000 samples per year for each year of the project total $6,358 and are detailed as follows: Superscript III one step kit ($4.62 per sample); Platinum Tag DNA Polymerase ($0.51 per sample); nuclease-free water ($0.15 per sample); and Axygen Pipette Tips and Filter Tubes ($1.08 per sample).

DNA Sequencing
In each year of the project, DNA Sequencing will be performed on 3,200 samples at a cost of $2.62 per reaction. We request a total of $2,601 per year in year.

Cell Culture
We request a total of $4,913 in year one; $6,647 in year two (upon expectation of 35% increase in positive samples); and $8,671 (a 30% increase) per year for the remaining years (3-5) of the project to cover costs for cell culturing. This will require GIBCO Fetal Bovine Serum, antibiotic antimycotic, growth medium, and cell culture plates and flasks. Flask costs are estimated at $1.45 per plate/flask.

Protein Expression and Purification

For protein expression and purification, we request a total of $6,590 in year 1, $6,705 in year 2, and $5,260 in each year of years 3-5. Details of protein expression and purification costs include histidine and sepharose tagged protein purification fast flow columns ($116 each), Q sepharose fast flow media ($116 each), protease inhibitor ($289 each), eStain Protein staining kits ($145 each), protein G sepharose 4 fast flow ($145 each), Q and SP sepharose fast flow ($723 each), and bacterial culture plates ($116 each). It is expected that more samples will be processed in year 1, so costs are estimated to reduce by 20% in years 2-5

Serological Tests

We request support for serology assays. None will be conducted in year one until we have samples. Costs are estimated for years 2-5 with $9,220 in year 2 and due to additional IgM and IgG secondary antibodies costs and increase in estimate to $9,436 in years 3-5. Cost estimates include IgM and IgG secondary antibodies at $72.25; mouse and rabbit IgG antibodies at $434; mouse and rabbit IgM antibodies at $434; and ELISA plates at $2.90 each.

Lab Supplies

Funding is requested to support laboratory supplies including three (3) -80˚C freezers ($4,340 each); reagents including agarose, sodium chloride, yeast extract, phosphate buffer, Tris and other biochemical reagents (average of $307 per year); centrifuge tube costs are estimated to increase in years 2-5 (average of $665 per year); disposable personnel protective equipment (PPE), portal breathing apparati (PAPR), globes and protective clothing to be used in BSL settings are estimated at $723 per year; cell lines will be required in years 1 and 2 ($867 each); and *in vitro* culture (lipofectamine2000) will be required at cost of $434 in year 1 and $2,168 per year in years 2-5.

H. Indirect Costs

We are requesting an extremely low indirect cost of 8% on all direct costs.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		85,000.00
Section B, Other Personnel		0.00
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		85,000.00
Section C, Equipment		0.00
Section D, Travel		21,570.00
1. Domestic	0.00	
2. Foreign	21,570.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		243,430.00
1. Materials and Supplies	243,430.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		350,000.00
Section H, Indirect Costs		28,000.00
Section I, Total Direct and Indirect Costs (G + H)		378,000.00
Section J, Fee		0.00
Section K, Total Costs and Fee (I + J)		378,000.00

Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Category	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	515,358	515,358	515,358	515,358	515,358	**2,576,790**

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ○ Yes ● No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

○ Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ❍ Yes ● No

If the answer is "Yes" then please answer the following:

*Previously Reported: ❍ Yes ❍ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	NIAID_COV_2019_SPECIFIC_AIMS_final.pdf
3. Research Strategy*	NIAID_COV_2019_RESEARCH_STRATEGY_final.pdf
4. Progress Report Publication List	NIAID_COV_2019_PROGRESS_REPORT_PUBLICATION_LIST.pdf
Other Research Plan Section	
5. Vertebrate Animals	NIAID_COV_2019_VERTEBRATE_ANIMALS_Final.pdf
6. Select Agent Research	NIAID_COV_2019_SELECT_AGENTS_Final.pdf
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	NIAID_COV_2019_CONSORTIUM_CONTRACTUAL_Final.pdf
9. Letters of Support	NIAID_COV_2019_LOS_Final.pdf
10. Resource Sharing Plan(s)	NIAID_COV_2019_RESOURCE_SHARING_PLAN_Final.pdf
11. Authentication of Key Biological and/or Chemical Resources	NIAID_COV_2019_AUTHENTICATION_OF_KEY_BIO_RSCS.pdf
Appendix	
12. Appendix	

SPECIFIC AIMS

Zoonotic coronaviruses are a significant threat to global health, as demonstrated with the emergence of Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) in 2002, and the continuing spread of Middle East Respiratory Syndrome (MERS-CoV). The wildlife reservoirs of SARS-CoV were identified by our group as bat species, and since then we have sequenced dozens of novel SARS-related CoV (SARSr-CoV) strains. Our previous R01 work demonstrates that bats in southern China harbor an extraordinary diversity of SARSr-CoVs, some of which are able to use human ACE2 to enter into human cells, can infect humanized mouse models to cause SARS-like illness, and evade available therapies or vaccines. We found that the bat hosts of SARSr-CoVs appear to no longer be traded in wildlife markets, and that people living close to bat habitats are the primary risk groups for spillover. At one of these sites, we found diverse SARSr-CoVs containing every genetic element of the wild-type SARS-CoV genome, and serological evidence of human exposure among people living nearby. Thus, there is significant potential for future spillover of SARSr-CoVs, and of public health impacts. *Yet salient questions remain:* *Are there specific bat communities and sites that harbor CoV strains with higher risk for bat-to-human spillover? Which human behaviors drive risk of bat SARSr-CoV exposure that could lead to infection? Does human exposure to these viruses cause SARS-like or other illness? Can we characterize viral strain diversity, bat traits and human behaviors to assess risk of potential future CoV spillover?* **The proposed work in this renewal R01 builds on these findings** to address these issues by conducting: **1)** focused sampling of bats in southern China to **identify viral strains with high predicted risk of spillover**; **2)** community-based, and clinic-based syndromic, sampling of people to **identify spillover, and assess behavioral risk factors and evidence of illness**; and **3)** conduct *in vitro* and *in vivo* viral characterization and analyze epidemiological data **to identify hotspots of future CoV spillover risk**. This work will follow 3 specific aims:

Aim 1: **Characterize the diversity and distribution of high spillover-risk SARSr-CoVs in bats in southern China.** We will conduct targeted bat sampling at sites where we predict that undiscovered high risk SARSr-CoV strains exist. Bat sampling will be targeted geographically and by host species to test predictions about evolutionary diversity of SARSr-CoV. We will analyze RdRp and S protein sequences to test their capacity for spillover to people in Aim 3.

Aim 2: **Community- and clinic-based surveillance to capture SARSr-CoV spillover, routes of exposure and potential public health consequences**. We will conduct focused, targeted human surveys and sampling to identify key risk factors for SARSr-CoV spillover and evidence of illness. To maximize our opportunity of capturing human exposure to bat CoVs, we will conduct community-based surveillance in regions with high SARSr-CoV prevalence and diversity, and individuals having contact with bats. We will assess bat-CoV seropositive status against a small number of questions about human-wildlife contact and exposure. We will conduct clinic-based syndromic surveillance close to these sites to identify patients presenting with influenza-like illness and severe acute respiratory illness, assess their exposure to bats via a questionnaire, and test samples for PCR- and serological evidence of SARSr-CoV infection. We will conduct follow-up sampling to capture patients who had not yet seroconverted at the time of clinic visit.

Aim 3: ***In vitro* and *in vivo* characterization of SARSr-CoV spillover risk, coupled with spatial and phylogenetic analyses to identify the regions and viruses of public health concern.** We will characterize the propensity of novel SARSr-CoVs to infect people *in vitro* using primary human airway epithelial cells and *in vivo* using the transgenic hACE2 mouse model. We will use mAb and vaccine treatments to test our hypothesis that SARSr-CoVs with 10-25% divergence in S protein sequences from SARS-CoV are likely able to infect human cells, and to evade mAb therapeutics and vaccines. We will then map the geographic distribution of their bat hosts and other ecological risk factors to identify the key 'hotspots' of risk for future spillover**.**

Overall, our SARSr-CoV program serves as a model platform to integrate virologic, molecular and ecologic factors contributing to CoV emergence while informing high impact strategies to intervene and prevent future pandemics. This includes providing critical reagents, therapeutic interventions and recombinant viruses for future SARSr-CoV pandemic and public health preparedness.

1. RESEARCH STRATEGY

A. Significance:

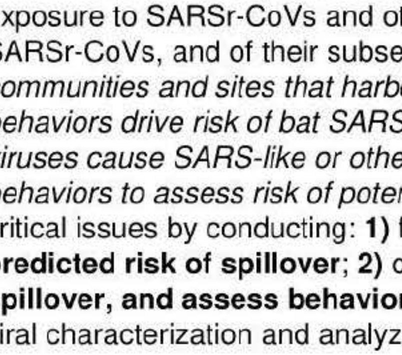
Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) emerged in China threatening public health and global economies (*1, 2*). Like most other emerging pathogens (*3*), it originated in animal reservoir hosts, initially thought to be carnivores (*4*), and later shown by our group to be bats (*5*). Bats harbor a high diversity of β-coronaviruses, including those related to Middle Eastern Respiratory Syndrome coronavirus (MERS-CoV) (*6-9*) and the newly emerged Swine Acute Diarrhea Syndrome coronavirus (SADS-CoV) (*10*), and may be the progenitor hosts of all *Coronaviridae* (*5, 11-15*). SARS-CoV uses the angiotensin-converting enzyme 2 (ACE2) receptor to gain entry to human cells (*16*). In 2012, we isolated and characterized two bat SARS-related coronaviruses (SARSr-CoVs) in China that use the ACE2 receptor and are closely related to SARS-CoV (*17*). Since then, under an R01 awarded in 2014, we have discovered >50 bat SARSr-CoVs in southern China. Some of these strains can bind to and infect human cells, cause SARS-like clinical signs in a humanized mouse model, and evade therapeutic and vaccine candidates against SARS-CoV (*18*). The *Rhinolophus* spp. bat hosts of these viruses are abundant across southern China, where hunting and consumption of wildlife is common and human population growth high, and where we have now identified serological evidence of exposure to SARSr-CoVs and other bat CoVs (*19*). Thus, there is significant potential for future spillover of SARSr-CoVs, and of their subsequent spread. *Yet salient questions remain: Are there specific bat communities and sites that harbor CoV strains with higher risk for bat-to-human spillover? Which human behaviors drive risk of bat SARSr-CoV exposure that could lead to infection? Does human exposure to these viruses cause SARS-like or other illness? Can we characterize viral strain diversity, bat traits and human behaviors to assess risk of potential future CoV spillover?* **This R01 renewal** proposal aims to address these critical issues by conducting: **1)** focused sampling of bats in southern China to **identify viral strains with high predicted risk of spillover**; **2)** community-based, and clinic-based syndromic, sampling of people to **identify spillover, and assess behavioral risk factors and evidence of illness**; and **3)** conduct *in vitro* and *in vivo* viral characterization and analyze epidemiological data **to identify hotspots of future CoV spillover risk**.

B. Progress report: R01 AI110964, Daszak PI, Project Period: 06/01/2014 – 05/31/2019

The aims of our previous R01 were to: 1) Assess bat SARSr-CoV spillover potential at high risk human-wildlife interfaces, e.g. the wildlife trade, as reported for the 2003 outbreak (*4, 20*); 2) Analyze how viral diversity and phylogeny relates to host range and risk of emergence; and 3) Use binding assays, cell culture and mouse models to test the propensity of different SARSr-CoVs to infect humans. We made significant discoveries leading to **18 published peer-reviewed papers (*18, 19, 21-33*), including two papers in *Nature* (*10, 34*), and a review in *Cell* (*35*)** (see Progress Report Publication List). These findings include:

Diversity and distribution of bat β- and SARSr-CoVs in Southern China.

We sampled and PCR-screened >16,000 individual bats from 6 families (16 genera) in southern China, finding 9 species positive (5,730 individuals screened) for SARSr-CoVs **(Table 1, Fig. 1)**. We identified 178 novel β-CoVs, of which 172 were novel (52 novel SARSr-CoVs). This includes members of a new β-CoV clade, "lineage E" (*26*), and diverse HKU3-related CoVs (179 sequences) within a 'sister' clade to the SARS-CoV lineage.



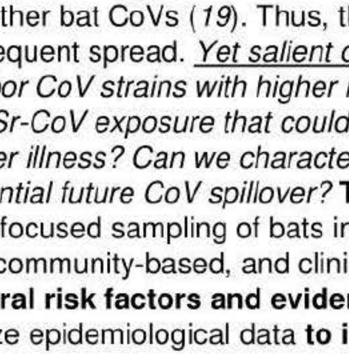


Fig. 1 (left): Bat sampling at 47 sites in China under our previous R01. Yellow = sampling effort, red = CoV +ve bats. **Fig. 2 (right)**: Map of bat species found positive for SARSr-CoVs in our previous R01, highlighting S. China (particularly Yunnan Province) as a center of diversity for SARSr-CoV reservoir host species.

We found 6.7% mean PCR prevalence of SARSr-CoVs across bat hosts, with a small number of *Rhinolophus* spp. horseshoe bats having significantly higher PCR prevalence than other species sampled **(Table 1)**. These bats are widely distributed, diverse, abundant, and roost and feed close to people and livestock, suggesting high potential for future SARSr-CoVs spillover. Distribution data for SARSr-CoV bat hosts suggest viral strain diversity is likely highest in southern China, particularly Yunnan Province **(Fig. 2)**.

Table 1: Species found PCR-positive for SARSr-CoVs in our R01, with sample sizes and prevalence estimates.

Bat Species	Individuals tested	# positive	SARSr-CoV mean prev.	SARSr-CoV prev. range
Rhinolophus sinicus	1,328	113	8.5%	7.1 – 10.1%
R. macrotis	70	3	4.3%	0.9 - 12%
R. ferrumequinum	406	12	3.0%	1.5 – 5.1%
R. spp.	331	10	3.0%	1.5 – 5.5%
R. affinis	792	7	0.9%	0.4 – 1.8%
R. pusillus	1,023	8	0.8%	0.3 – 1.5%
Aselliscus stoliczkanus	269	2	0.7%	0.1 – 2.7%
Hipposideros pratti	323	2	0.6%	0.1 – 2.2%
H. armiger	1,188	1	0.1%	0.0 – 0.5%

We identified one cave system (the "Jinning Cave") in Yunnan Province that harbors *Rhinolophus* spp. bats with diverse SARSr-CoVs, including some with S proteins able to use human ACE2 as entry receptors. Bats in this cave carried SARSr-CoVs with **all unique genetic elements of the SARS-CoV outbreak virus**, suggesting that this site may be a potential public health risk (*29*).

We used a novel phylogeographic analysis, Maximum Clade Credibility (MCC) tree, to reconstruct the geographic areas of evolutionary origin for β-CoVs that we sequenced. Results suggest that: 1) Guangdong Province is the ancestral center of diversity of β-CoVs (data not shown); 2) Guizhou is the likely origin of the HKU3-related clade (lineage 1); and 3) Guangdong and Guangxi are the likely ancestral origins of the SARS-CoV outbreak sequences (lineage 2) **(Fig. 3)**. Despite our intensive sampling at some sites, around half of the 20 *Rhinolophus* spp. we identified were captured at sample sizes below the minimum required to detect SARSr-CoVs at prevalences we found (n=110, power 80%), and 5 others were SARSr-CoV negative in our study. To estimate sampling gaps, we used a viral 'mark-recapture' approach we previously published (*36, 37*). Results suggest we are approaching saturation of CoV strain discovery at some sites, whereas other sites contain rich pools of SARSr-CoVs that remain undiscovered **(Fig. 4)**. **In the current proposal,** we have used these analyses to estimate geographic and species-specific sampling targets **to more effectively identify new strains and CoV lineages needed to support experimental infection studies and risk assessment.**

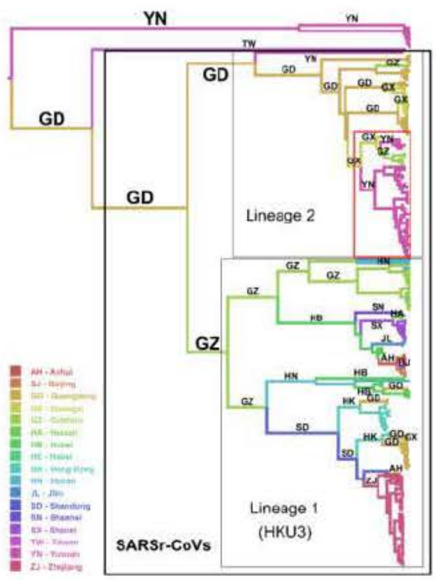


Fig.3 (left): MCC phylogeny of lineage B β-CoVs, including SARSr-CoVs (black box). Lineage 1 includes HKU3-related CoVs, lineage 2 includes SARS-CoV outbreak strains and close relatives (red box). Branches colored according to province of inferred ancestral origin (Guangdong GD, Yunnan YN, Guizhou, GZ).

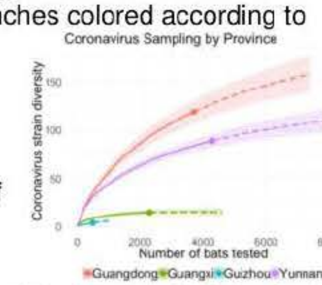


Fig. 4 (right): Estimates of SARSr-CoV strain diversity in the bats we sampled (strain defined as >10% sequence divergence in RdRp gene). GD and YN harbor highest CoV diversity, but discovery has not yet saturated. We estimate proposed additional sampling of 5,000 bats will identify >80% of remaining β-CoV strains in bat hosts from these regions.

<u>In vitro & in vivo *characterization of SARSr-CoV potential for human infection*</u>

We conducted *in vitro* and *in vivo* experiments to characterize the pathogenic potential of novel SARSr-CoVs. We isolated three SARSr-CoVs from bat feces: WIV1, WIV16 and Rs4874, with S protein sequences that diverged from SARS-CoV by 3% to 7% (*17, 22, 29*). We conducted full-length genome sequencing of 12 other novel SARSr-CoVs from the Jinning Cave, some highly similar to outbreak SARS-CoV in the most variable genes: N-terminal domain and receptor binding domain (RBD) of the S gene, ORF8 and ORF3 (*29*). Using our reverse genetics system, we constructed chimeric viruses with SARSr-CoV WIV1 backbone and the S gene of different variants, including WIV1-Rs4231S and WIV1-Rs7327S. All 3 SARSr-CoV isolates and the two chimeric viruses replicated efficiently in Vero E6 cells and in HeLa cells expressing hACE2, but not in HeLa cells that don't express ACE2 (*17, 22, 29*) **(Fig. 5a)**. In collaboration with Ralph Baric (UNC), we used the SARS-CoV reverse genetics system (*38*) to generate a chimeric virus with a mouse-adapted SARS-CoV

backbone expressing SHC014 S protein with 10% sequence divergence from SARS-CoV S. This chimera replicated in primary human airway epithelium, using the human ACE2 receptor to enter into cells (*18*) (**Fig. 5b**). Thus, **SARSr-CoVs with diverse variants of SL-CoV S protein without deletions in their RBD can use human ACE2 as receptor for cell entry.**

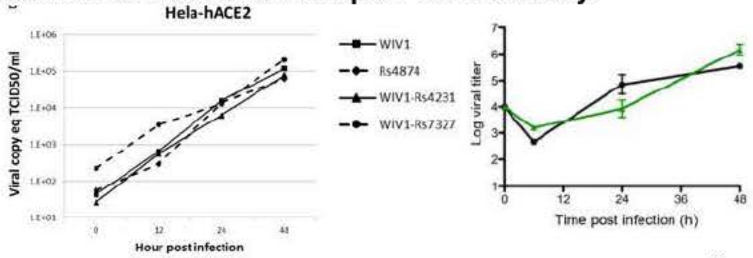


Fig. 5a (left): RT-PCR shows that bat SARSr-CoVs WIV1, Rs4874, and chimeras WIV1-Rs4231S, WIV1-Rs7327S grow in HeLa cells expressing human ACE2. **Fig. 5b (right):** Viral replication of SARS-CoV Urbani (black) and SARS-SHC014S (green) primary air-liquid interface human airway epithelial cell cultures at an MOI of 0.01.

We infected transgenic mice expressing hACE2 with 10^5 pfu of full-length recombinant WIV1 and three chimeric viruses (WIV1 backbone with SHC014S, WIV16S and Rs4231S). hACE2 transgenic mice challenged with rWIV1-SHC014S experienced ~20% body weight loss by 6dpi; rWIV1 and rWIV-4231S produced less body weight loss, and rWIV1-WIV16S led to no body weight loss **(Fig. 6a)**. At 2 and 4 dpi, viral loads in lung tissues of mice challenged with all three chimeras reached > 10^6 genome copies/g, significantly higher than rWIV1 infection **(Fig. 6b)**. This demonstrates that pathogenicity of SARSr-CoVs in humanized mice differs with divergent S proteins, **confirming the value of this model in assessing novel SARSr-CoV pathogenicity.**

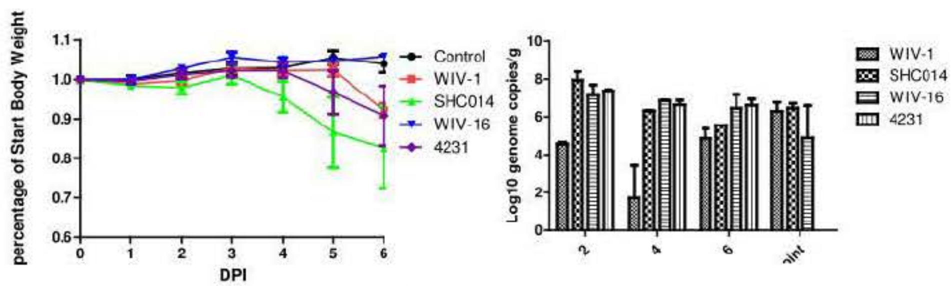


Fig. 6: *In vivo* infection of SARSr-CoVs in hACE2 transgenic mice. **6a (left)** Body weight change after infection; **6b (right)** Viral load in lung tissues.

Infection of rWIV1-SHC014S caused mild SARS-like clinical signs in the transgenic hACE2 mouse model **that weren't reduced by immune-therapeutic monoclonals that attenuate SARS-CoV pathogenecity.** Vaccination against SARS-CoV did not reduce severity of clinical signs in mice subsequently infected with rSARS-SHC014S (*18*). We found 2/4 broad human mAbs against SARS-CoV RBD cross-neutralized WIV1, but none could efficiently neutralize SHC014 which is less similar to SARS-CoV in the RBD (*39*). We repeated this virus characterization approach with chimeras using HKU3r-CoV S proteins that are ~25% divergent from SARS-CoV S, **and found that they are unable to use the ACE2 receptor**. Additionally, we were unable to culture HKU3r-CoVs in Vero E6 cells, or human cell lines. **The ability of HKU3r-CoVs to infect people, and their receptor binding target, remain unknown.**

This work has three implications for our R01 renewal: 1) some SARSr-CoVs currently circulating in bats in southern China **are likely able to infect and replicate within people**; 2) clinical outcomes of infection may include SARS-like illness that is currently **not treatable with mAb nor preventable with experimental vaccines**; 3) SARSr-CoV ability to bind human ACE2 is lost with S protein divergence between 10% (SHC014) and 25% (HKU3r-CoVs). **Although no viruses within this range have so far been described, these strains likely use hACE2 but could escape existing vaccines and immunotherapeutics and represent significant public health threats**. In our R01 renewal proposal, we will actively seek to identify viruses with this level of S protein divergence, characterize their binding targets *in vitro*, and their capacity to produce SARS-like disease that evades immunotherapy and vaccination *in vivo*.

Discovery of a novel bat-origin α-CoV associated with pig die-offs

Coronaviruses have a well-described propensity to jump the species barrier and cause new outbreaks (*40*). In 2016-17, we analyzed fecal samples from pigs at 5 farms in Guangdong Province (GD) affected by a fatal diarrheal disease. We discovered an α-CoV closely related to HKU2, and used PCR, serological and pathological data, followed by infection experiments to demonstrate that this novel virus, Swine Acute Diarrheal Syndrome coronavirus (SADS-CoV), caused the death of more than 20,000 pigs at these farms (*10*). We

identified SADSr-CoVs in *Rhinolophus* spp. bats in GD, and analyzed >30 full-length genomes to provide phylogenetic evidence that SADS-CoV originated in these bats (**Fig. 7**).

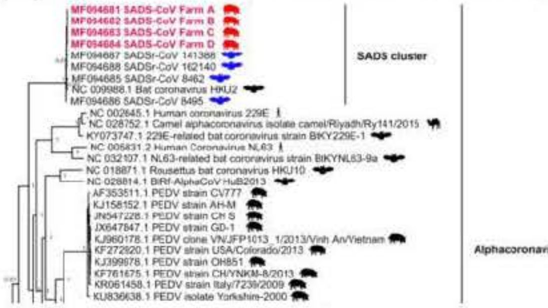


Fig. 7: Bayesian phylogenetic tree of the full-length genome sequences of SADS-CoV (red), bat SADSr-CoVs (blue), and related α-coronaviruses. Host species represented by symbol.

SADS-CoV replicates in Vero cells (*10, 41, 42*), but its capacity to replicate in human cell lines, and its zoonotic potential remains unknown. We developed a novel Luciferase Immunoprecipitation Systems (LIPS) antibody assay for SADS-CoV and found no evidence of spillover to pig farm workers at affected farms (0/33 people seropositive) (*10*). In the current proposed work we will include SADS-CoV diagnostic reagents in our serological panel to opportunistically screen human samples for evidence of spillover into people exposed to bats in southern China

Mapping bat viral emergence risk

We analyzed host and viral data for all known mammalian viruses and used a generalized additive models to correct for underlying sampling and reporting biases (*34*). This approach allowed us to predict the relative number of yet-to-be-described or 'missing' viruses that a species likely harbors. For China, there are distinct hotspots of unknown bat viral diversity in Yunnan Province **(Fig. 8)**.

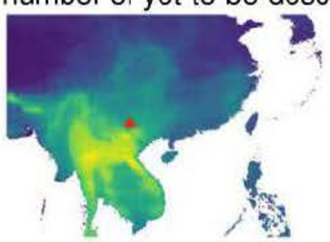


Fig. 8: Spatial distribution of predicted 'missing' or as-yet undiscovered viruses, from (*34*). Yellow = highest diversity, red triangle = Jinning Cave, Yunnan (*29*).

In a separate paper, we found that bat host diversity and climatic variability are correlates of viral diversity within bats, and that human population density, bushmeat hunting, and livestock production are correlates of the risk of transmission for viruses that spillover (*26*). The risk of spillover and spread differ spatially, suggesting that locations where bat viruses are most diverse may not be the most strategic sites for public health intervention (*21*). Work in the current proposal will improve on both approaches to identify hotspots of CoV emergence risk, by **using data from the high-risk locations and interfaces identified in our previous R01**, including better characterization of SARSr-CoV diversity in bats, and the potential of these viruses to cause infection.

Human risk behavior, the wildlife trade, and evidence of bat SARSr-CoV spillover.

Qualitative Study: Our previous R01 hypothesis was that SARSr-CoV spillover would most likely occur through the trade in bats for food, via the same market chains that to the emergence of SARS (*20*). To test this, we conducted an exploratory study using standardized one-on-one semi-structured ethnographic interviews and observational data in southern China among 88 people involved in trading wild bats, to assess local social and cultural norms and individual attitudes underlying contact with bats (publication in prep.). Our results suggest that in the 11 years since the emergence of SARS, there have been substantial changes to the wildlife trade: 1) Former wildlife markets are now predominantly selling captive-bred species (poultry, livestock, farmed wildlife); and 2) few bats are now sold through markets. We identified other risk factors for spillover, including people living near to bat roosts, and those visiting bat caves for hunting or recreation.

Human Questionnaire & Sero-surveillance: We used qualitative study findings to develop a human behavioral risk questionnaire on the type and frequency of animal contact, wildlife observed in daily life, and unusual illnesses reported over the past 12 months. We conducted a cross-sectional study among populations that live near bat caves or roosts where we had detected bat SARSr-CoVs. Study participants provided biological samples, and bats were concurrently captured and sampled. Questionnaires and biological samples (oropharynx swab, serum, plasma) were collected from 1,585 participants from 7 sites in Yunnan, Guangxi, and Guangdong provinces **(Fig. 9)**.

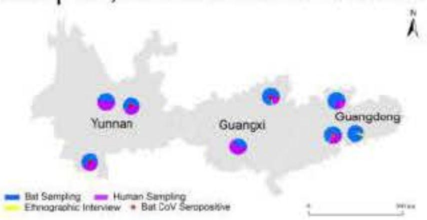


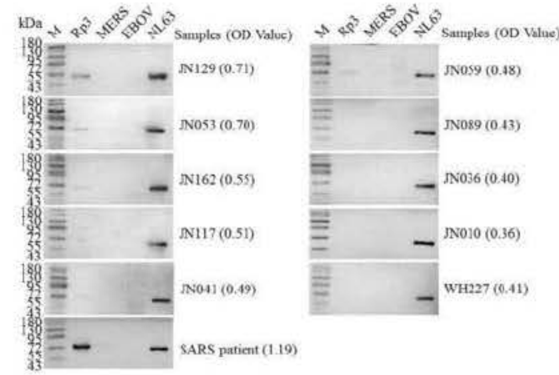
Fig. 9: Concurrent sampling in bats and target human population in communities in Yunnan, Guangxi, and Guangdong provinces. Pie-charts indicate sampling effort (bat sampling = blue, Human questionnaire and sampling = purple, Ethnographic interview = yellow, bat CoV seropositive = red)

Serological Evidence of Exposure: We developed serological assays for HKU9 CoVs (β), SARSr-CoV Rp3 (β), HKU10 CoV (α), and MERS-CoV (β) and used ELISA and Western blot to test serum samples collected in 2016/17. **We found 7 individuals (7/733, 0.95%) living within a 6 km radius of the Jinning Cave, and 6/209 people (2.87%) at one site, with evidence of exposure to bat SARSr-CoVs (Table 2; Fig. 8).**

Site	# tested	Bat CoV + (%)	SARSr-CoV Rp3 + (%)	HKU10 + (%)	HKU9 + (%)	MERS-CoV+ (%)
Jinning, Yunnan	209	6 (2.87)	6 (2.87)	-	-	-
Mengla, Yunnan	168	1 (0.6)	1 (0.6)	-	-	-
Jinghong, Yunnan	212	-	-	-	-	-
Lufeng, Yunnan	144	-	-	-	-	-
Guangdong	420	-	-	-	-	-
Guangxi	412	2 (0.48)	-	2 (0.48)	-	-

Table 2 (left): ELISA and Western blot confirmed testing of human sera for antibodies to 4 bat CoVs.

Fig. 10: Western blot reactivity of human sera to SARSr-CoV Rp3 NP.



We found evidence among human populations in Guangxi Province of people with prior exposure to the bat α-CoV HKU10 (2/412, 0.48%). This is of potential public health interest because HKU10 is known to be able to jump host species within bats, and therefore may have high propensity for emergence (*43*).

Behavioral Risk Factors: Questionnaire response and demographic data suggest specific risk factors included type of occupation, keeping of pets and visiting wet markets. Seropositive individuals were mostly farmers/peasants (8/9), living in Yunnan province (7/9), 41-60 yrs old (7/9), with domestic contact with rodents (6/9), and are male (6/9). However, these characteristics were common and occurred in ~40% of survey respondents. Although these results are preliminary and don't provide detailed information on routes of exposure, they suggest that further refining use serological tests coupled with qualitative and questionnaire data will identify likely routes of exposure to novel CoVs in China. In Aim 2 of this R01 renewal proposal, we identify strategies to better target at-risk people, and conduct focused **questionnaires and serosurveys to produce statistically significant findings.**

Analysis of self-reported illness: We analyzed data on self-reported symptoms of fever with cough and shortness of breath or difficulty breathing (severe acute respiratory illness - SARI), and fever with muscle aches, cough, or sore throat (influenza-like illness - ILI) from study participants. We used a least absolute shrinkage and selection operator (LASSO) regression to identify associations between ILI and/or SARI symptoms and contact with animals in the last year. Salient predictors or combination of predictors were, in descending order of odds ratio: 1) having eaten raw or undercooked carnivores; 2) having slaughtered poultry and being a resident of Guangxi province; 3) having had contact with cats or bats in the house; and 4) being a male who has raised poultry **(Fig. 11).**

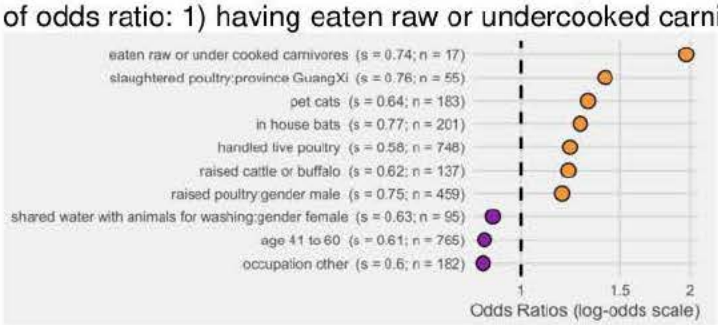


Fig. 11: Predictors of self-reported ILI and/or SARI in prior 12 months (s = bootstrap support; n = number +ve out of 1,585 respondents). Orange circles = odds ratios > 1 (positively associated with the outcome); purple = odds ratios <1 (negatively associated with the outcome).

2. APPROACH

Rationale and Innovation : Our previous R01 work demonstrates that bats in southern China harbor an extraordinary diversity of SARSr-CoVs, some of which are able to use human ACE2 to enter into human cells, can infect humanized mouse models to cause SARS-like illness, and evade available therapies or vaccines. We found that the bat hosts of SARSr-CoVs appear to no longer be traded in wildlife markets, and that people living close to bat habitats are the primary risk groups for spillover. At one of these habitats, we found diverse SARSr-CoVs containing every genetic element of the wild-type SARS-CoV genome, and serological evidence of human exposure among people living nearby. The proposed work in this renewal R01 builds on these

findings:. **In Aim 1, we will conduct targeted bat sampling at sites where we predict that undiscovered high risk SARSr-CoV strains exist.** Bat sampling will be targeted geographically and by host species to test predictions about evolutionary diversity of SARSr-CoV. We will analyze RdRp and S protein sequences to test their capacity for spillover to people in Aim 3. **In Aim 2,** will conduct focused, targeted human surveys and sampling **to identify key risk factors for SARSr-CoV spillover and evidence of illness.** To maximize our opportunity of capturing human exposure to bat CoVs, we will conduct community-based surveillance in regions with high SARSr-CoV prevalence and diversity, and individuals having contact with bats. We will assess bat-CoV seropositive status against a small number of questions about human-wildlife contact and exposure. We will conduct clinic-based syndromic surveillance close to these sites to identify patients presenting with influenza-like illness and severe acute respiratory illness, assess their exposure to bats via a questionnaire, and test samples for PCR- and serological evidence of SARSr-CoV infection. We will conduct follow-up sampling to capture patients who had not yet seroconverted at the time of clinic visit. **In Aim 3,** we will characterize the propensity of novel SARSr-CoVs to infect people *in vitro* using primary human airway epithelial cells and *in vivo* using the transgenic hACE2 mouse model. We will use mAband vaccine treatments to test our hypothesis that SARSr-CoVs with 10-25% divergence in S protein sequences from SARS-CoV are **likely able to infect human cells, and to evade mAb therapeutics and vaccines**. We will then map the geographic distribution of their bat hosts and other ecological risk factors to **identify the key 'hotspots' of risk for future spillover.** Our SARSr-CoV program serves as a model platform to integrate virologic, molecular and ecologic factors contributing to CoV emergence while informing high impact strategies to intervene and prevent future pandemics. This includes providing critical reagents, therapeutic interventions and recombinant viruses for future SARSr-CoV pandemic and public health preparedness.

Research team and management: We have reinforced our original collaboration between EcoHealth Alliance (EHA), a global leader in field investigations of emerging viruses from wildlife and modeling/analysis of viral risk, and Wuhan Institute of Virology, a global leader in bat viral investigations **(Fig. 12)**. First, we have included senior behavioral risk scientists Co-I Francisco (EHA) and Ren (Inst. Pathogen Biol., Beijing) to oversee human survey and sampling work in Aim 2. Second, Prof. Linfa Wang (Duke-NUS), a world leader in understanding the role of bats as hosts of emerging viruses, will act as a consultant by advising and assisting in the development of PCR and serological tests and virus characterization. Prof. Wang has developed a unique array of bat immunological reagents that enrich the serological arms of the proposal. Third, Prof Ralph Baric (UNC) will use his expertise in CoV characterization to conduct primary human epithelial airway cell infections to identify high risk strains that are poised for human emergence. He will oversee and participate in animal experiments in Aim 3. This expanded team will work on a more focused set of goals, based on the results of our previous R01. PI Daszak has collaborated with all partners for between 3 and 15 yrs and will host monthly calls, annual in-person meetings, conduct quarterly adaptive management to refine research lines of work.

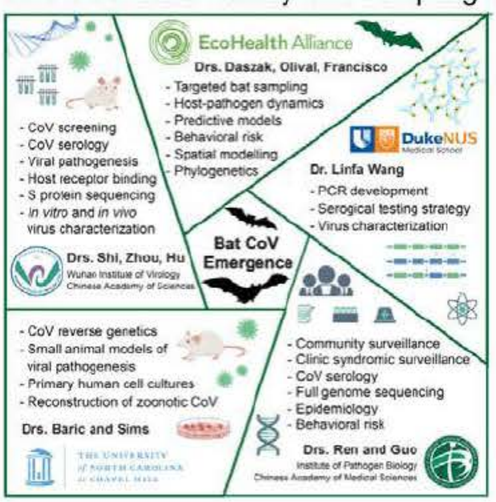


Fig. 12: Interdisciplinary team & roles in the proposed R01 renewal work.

Aim 1: Characterize the diversity and distribution of high spillover-risk SARSr-CoVs in bats in southern China

1.1 Rationale/Innovation: Our previous R01 work identified diverse SARSr-CoVs with high propensity for human infection (*18, 19, 29*). Characterization of these suggests SARSr-CoVs that are up to 10%, but not 25% different in the spike glycoprotein use human and bat ACE2 receptors for docking and entry. Uneven sampling gaps (e.g., no strains were found with 10-25% spike variation) prevent a thorough understanding of the transition point where the most divergent strains lose human ACE2 receptor usage. Our viral discovery curves **(Fig. 4)** suggest further sampling will reveal a rich diversity of as-yet-undiscovered SARSr-CoVs. In this aim we will use phylogenetic and viral discovery analysis to specifically target bat species and regions that are undersampled to allow sufficient power to identify and characterize missing SARSr-CoV strain diversity. Our

previous work also suggests that SARSr-CoVs with S proteins that are ~10% divergent from SARS-CoV resist neutralization by therapeutic mAbs and escape SARS-CoV vaccines (*17, 18, 23*), suggesting that . However, viruses with 10-25% divergence in S proteins may bind to human cell receptors, but completely evade therapeutic and vaccine effects, and could therefore be a higher risk for public health. We will sequence the S proteins of novel SARSr-CoVs to prioritize viruses for experimental work in Aim 3 to test this hypothesis.

1.2 General Approach: We will use sampling, testing, and CoV sequence data from our previous R01 to pinpoint sites and host species needing additional sampling. We will work in 4 provinces (Yunnan, Guangxi, Guizhou and Guangdong) that we have identified phylogenetically as having the highest diversity of as-yet-undiscovered SARSr-CoVs and with competent natural hosts. Precise sampling site locations will be refined in Y1. We will target at least 5,000 individual bats over 5 years from 15 currently undersampled species of *Rhinolophus* bats, which we calculate will allow us to almost fully characterize the expected natural diversity of SARSr- and other β-CoVs in the region. Bats will be captured, sampled, and released at the site. Specimens will be transported in liquid N_2 to Wuhan Inst. Virology (WIV) for PCR screening, and positive samples selected for further molecular characterization and S Protein sequencing. EHA will lead the study design, field sampling, and data analysis for this Aim; and WIV will lead the testing and viral sequencing.

1.3 Sampling and testing of bats: 1.3.a Site selection & sample sizes: In Y1 we will use our bat host and viral trait modeling, phylogeographic analyses of RdRp and S Protein sequences, and geographic and host species-based viral discovery curve analyses to identify SARSr-CoV diversity hotspot regions for bat sampling. We will sample at 8 new sites in four provinces. We will use cave site data (*44*), and demographic information to identify two sites in each of Yunnan, Guangxi, Guangdong, and Guizhou where humans likely have contact with bats. In Yunnan, we will identify two unsampled caves close to, but distinct from, the Jinning cave (*29*). This will provide adequate coverage of lineage 1 and 2 SARSr-CoVs, including a rich source of new HKU3r-CoVs, which have unknown potential for zoonotic spillover. Sampling will begin towards the end of Y1. We will use survey data from our previous R01 and host-specific viral accumulation curve data to target an additional 10 under-sampled *Rhinolophus* spp., 5 that were SARSr-CoV negative in our study, and a small number of related bat genera (including *Hipposideros* spp. and *Aselliscus* spp.) we previously found PCR positive for SARSr-CoVs **(Table 1)**. We will sample at least 5,000 bats from these 4 provinces (~1250 per province). Given ~5-12% prevalence of SARSr-CoVs in *Rhinolophus* spp. at our previous sites, **this sample size would give us 425 (±175) positive individual bats, and ~125 novel strains.**

1.3.b CoV screening, isolation: Viral RNA will be extracted from bat fecal pellets/anal swabs with High Pure Viral RNA Kit (Roche). RNA will be aliquoted, and stored at -80C. One-step hemi-nested RT-PCR (Invitrogen) will be used to detect the presence of CoV sequences using primers that target a 440-nt fragment in the RNA-dependent RNA polymerase gene (RdRp) of all known α- and β-CoVs (*45*). PCR products will be gel purified and sequenced with an ABI Prism 3730 DNA analyzer. We will attempt isolation on samples with diverse and interesting novel CoVs, using Vero E6 cells and bat primary cell culture.

1.3.c Sequencing S proteins: For all novel SARSr-CoV strains, we will sequence the complete S gene by amplifying overlapping fragments using degenerate primers as shown previously (*17, 29*). Full-length genomes of selected SARSr-CoV strains (representative across subclades) will be sequenced via high throughput sequencing method followed by genome walking. The sequencing libraries will be constructed using NEBNext Ultra II DNA Library Prep Kit for Illumina and sequenced on a MiSeq sequencer. PCR will be performed to fill gaps in the genome. The full length S gene sequences, including the amount of variation in the S receptor binding residues that bind the ACE2 receptor, will be used to select strains for Aim 3 experiments.

1.3.d Host ACE2 receptors: We will sequence host ACE2 receptors of different bat species or different bat populations from a single species (e.g. *Rhinolophus sinicus*) to identify relative importance of different hosts of high risk SARSr-CoVs and the *intra*specific scale of host-CoV coevolution. Of particular interest is homology across 18 bat and human orthologue ACE2 contact interface residues that engage the SARS RBD as a potential indicator of SARSr-CoV cross species transmission potential and growth in human cells (*46*).

1.4 Analyses: 1.4.a Bat-CoV evolution and distribution: We will use Bayesian and Maximum Likelihood phylogenetic analyses of RdRp, Spike, and full genome sequence (when available) data to reconstruct the evolutionary history of the novel bat SARSr-CoVs we identify. We will rerun MCC analyses **(Fig. 3)** to

reconstruct β-CoV evolutionary origins using our expanded dataset. Ecological niche models will be used to predict spatial distribution of PCR-positive species, identify target sites for additional bat sampling, and analyze overlap with people. We will use our viral discovery/accumulation curve approach **(Fig. 4)** to monitor progress towards discovery of >80% of predicted diversity of SARSr-CoVs (lineage 1 & 2) within species and sites, halting sampling when this target is reached, and freeing up resources for work other work (*37, 47*).

1.4.b Viral strain prioritization: Of the expected 100-200 novel SARSr-CoV strains, we will down-select to prioritize for further characterization based on S genes that are: i) different from SHC014, WIV1, SARS-CoV with diversity ranges of 10-25%; ii) have virus S RBD that could use human/bat receptors; iii) have recombinant chimeric spikes indicative of gene flow between clade I and II strains; iv) have bat ACE2 receptors that might select for spike RBDs that can use human receptors for entry (15/18 conserved residues in human/bat ACE2 molecules that bind SARSs-CoV S RBD domains are likely more efficient receptors than 3/18 conserved sites). Using structural models based on the SARS S glycoprotein, the extent and location of antigenic variation will be annotated onto the structure, further resolving the locations of highly evolving and conserved structural motifs/epitopes that function in protective immunity (*48-51*).

1.5 Potential problems/alternative approaches: Permission to sample bats in sites or provinces we select. We have a >15-year track record of successful field work in southern China and have worked with local authorities and communities to ensure access. We have existing permissions in place which will be renewed at the start of this project. Due to the abundance of distinct caves in the region, if access to a site is withdrawn, we will rapidly identify a suitable, complementary site with similar bat species composition and abundance. **We may not identify β-CoVs in our sample bat species due to seasonality of viral shedding.** The sampling regions we have selected are subtropical, and our previous data, and even published studies in temperate regions (*52*), do not suggest a strong pattern of seasonality in SARSr-CoV shedding. Nonetheless to account for this we will conduct sampling evenly on quarterly basis within each province.

<u>Aim 2: Community and clinic-based surveillance to capture SARSr-CoV spillover, routes of exposure and potential public health consequences.</u>

2.1 Rationale: Our previous R01 study identified serological evidence of exposure to SARSr-CoVs in certain communities in S. China **(Table 2, Fig. 10)** (*19*). However, the low seroprevalence (0.6%-2.7% at positive sites) suggests we need a larger sample size, and a focused, targeted study to maximize the likelihood of identifying seropositive cases. In Aim 2, we will use combined biological-behavioral risk surveillance in targeted populations within the community and clinical settings to 1) identify risk factors correlated with seropositivity (exposure to) and PCR positive status (infection with) bat SARSr-CoVs; and 2) assess possible health effects of SARSr-CoVs infection in people. Obtaining this information could be a significant step in understanding the likelihood of recent 'hidden' spillover events and their public health impacts, as well as the risk of future emergence of SARS-like diseases. It will also support the development of risk-mitigation strategies by public health authorities within China and other countries with bats that harbor these viruses (e.g. most of SE Asia).

2.2 General Approach/Innovation: We will use a dual study design to gain in depth understanding of exposure and risk factors for SARSr-CoV spillover **(Fig. 13)**. We will conduct <u>community-based surveillance</u>, with more focused questionnaires and biological sampling to determine the seroprevalence of SARSr-CoVs in at-risk human populations, and to identify risk-factors for SARS-CoV spillover in these communities. We will conduct <u>clinic-based syndromic surveillance</u> and biological sampling at sites that include the community-based surveillance sites within their catchment. This will include follow-up sampling to capture seroconversion of recently infected people and the full course of symptoms. We will also use PCR to test for present of active SARSr-CoV replication. Both community-based and clinic-based syndromic surveillance programs are <u>case-control studies</u> designed with the sample sizes necessary to statistically quantify (with a power of 80%) risk factors and health impacts for SARSr-CoV spillover, linked to serological status and symptoms.

2.3 Target population & sample size: We will target sites in the same four provinces, and close to those for bat sampling, based on: 1) sites of likely divergence of SARSr-CoVs; 2) a high diversity of SARSr-CoVs within the S protein sequence divergence of 5-25%; and 3) high rates of human-wildlife interactions. <u>Community-based surveillance</u> will be conducted at 2 sites in each of the 4 provinces, a total of 8 sites. From our previous work we anticipate that 10-30% of the community population will have had exposure to bats allowing us to

capture highly exposed and non-exposure individuals at each site. Individuals living or working around bat roosts, who hunt wildlife, work with wildlife or livestock farming, transportation, selling, or slaughtering wildlife in the surveyed areas will be targeted so that they make up ≥30% of the sampled population in each community. We will stratify sampling to ensure appropriate representation of sex, demographic, and socio-economic factors at each community site. We will initiate active <u>clinic-based syndromic surveillance</u> at 2 county-level hospitals and 1 provincial-level hospital in each of the 4 provinces, in total 12 hospital sites, all within the catchment areas for bat sampling, and which are used by people in our community-based surveillance. Patients ≥ 12 years old presenting at the health facility who meet the syndromic and clinical case definitions for SARI and ILI will be recruited into the study. We will enroll a total of at least 2,750 individuals for clinical studies, which accounts for up to 40% loss from follow-up. Study data will be pooled across sites, as clinical patients are limited by the number of individuals presenting at hospitals. For community-based surveillance, we will enroll 1,650 individuals per province, pooled across two sites for each province, allowing us to make province-level comparisons of differing effects. Estimating 5% overall seroprevalence in these high-exposure populations, these sample sizes are sufficient to estimate effect sizes of behavioral risk factors of 2X or greater with 80% power.

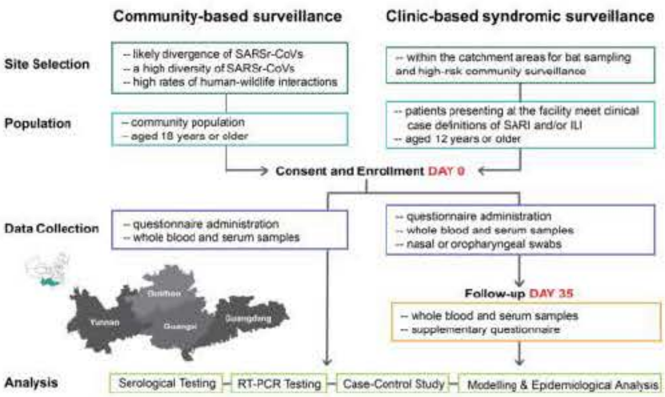


Fig. 13: Human survey and sampling study design overview

2.4 Data & sample collection: At both community and clinical settings, following enrollment with signed consent form, <u>biological specimens</u> (two whole blood samples, one max. 500 µL; two 500 µL serum samples) will be collected from all eligible participants, and a <u>questionnaire</u> will be administered. We will investigate five risk factors, so as to maximize the power of the analyses, all related to high risk wildlife exposure, based on continuing analysis of our previous work, and will include: 1) occupation; 2) observed or reported interactions with bats in/around house; 3) proximity to nearby bat roosts; 4) working or regular visit to animal markets; 5) self-reported ILI/SARI. An additional two nasal or oropharyngeal swabs will be collected from patients enrolled in the clinic-based syndromic study. With permission from each clinic, and consent from participants, we will review clinical records to collect data on medical history, clinical syndromes, and patient etiology.

2.5 Clinic enrollment and follow-up: We will recruit inpatients and outpatients after initial screening to meet the clinical case definition of 1) severe/acute respiratory illness (SARI/ARI) of unknown etiology; or 2) Influenza-like illness (ILI) of unknown etiology. Once enrolled, biological samples will be collected and a questionnaire administered by trained hospital staff that speak appropriate local dialects. Samples will be taken concurrently when collecting samples for normative diagnostics. For inpatients, samples will be collected within 10 days of reported onset of illness to increase the chance of PCR CoV detection (*53*). We will follow up 35 days after enrollment to collect an additional two 500 µL serum samples conduct a standardized questionnaire supplement to collect additional data on the course of symptoms in the interim period. 35 days gives adequate time for development of IgG, which occurs <28 days after onset of symptoms for SARS patients (*54*).

2.6: Laboratory analysis: 2.6.a Serological testing: In our previous R01, we expressed his-tagged nucleocapsid protein (NP) of SARSr-CoV Rp3 in *E.coli* and developed a SARSr-CoV specific ELISA for serosurveillance using the purified Rp3 NP. The specificity of this assay was evaluated using polyclonal antibodies against HKU1, OC43, 229E, NL63, MERS-CoV and EBOV and no significant cross-activity was detected (*19*). **While this shows it is a specific test for Rp3, it suggests that if we can expand our serology tests to cover other bat CoVs, we may identify many more seropositive individuals.** In this renewal, we will therefore use two serological testing approaches. First, we will expand test all human sera collected from both community- and clinic- based sampling for a panel of bat CoVs that will include SARS-CoV (outbreak strain), a range of lineage 2 SARSr-CoVs (including WIV1 and SHC014), lineage 1 HKU3r-CoVs, MERS-CoV, and the α-CoVs SADS-CoV and HKU10. We previously found serological evidence of human exposure to HKU10 (*19*), but HKU10 is known to jump from one host bat species to another (*43*) and is

therefore likely to have infected people more widely. Incoporating serological testing for SHC014 is also likely to yield higher seroprevalence because it is readily divergent from SARS-CoV wildtype and therefore unlikely to have been picked up in our earlier testing. It is possible that non-neutralizing cross reactive epitopes exist that afford an accurate measure of cross reactivity between clade1 and clade 2 strains which would allow us to target exposure to strains of 15-25% divergence from SARS-CoV. Additionally, we will test samples for antibodies to common human CoVs (HCoV NL63, OC43 – see potential pitfalls/solutions below). Secondly, we recognize that CoVs have a high propensity to recombine. To serologically target 'novel' recombinant virus exposure, we will conduct 1) ELISA screening with SARSr-CoV S or RBD; 2) confirm these results by Western blot; then 3) use NP based ELISA and LIPS assays with a diversity of SARSr-CoV NP. For NP ELISA, micro-titer plates will be coated with 100 ng/well of recombinant batCoV NP and incubated with human sera in duplicates followed by detection with HRP labeled goat anti-human IgG antibodies. For confirmation, all ELISA positive samples will be subjected to Western blot at a dilution of 1:100 by using batCoV-NP as antigen. We will use an S protein-based ELISA to distinguish the lineage of SARSr-CoV. As new SARSr-CoVs are discovered, we will rapidly design specific Luciferase immunoprecipitation system (LIPS) assays targeting the S1 genes of bat-CoV strains for follow-up serological surveillance, as per our previous work (*10*).

2.6.b RT-PCR testing. Specimens will be screened using RT-PCR for the RdRp gene (See section 1.3.b for details). Positive samples will be subjected to full genome sequencing and RT-PCR amplification of the S glycoprotein gene. Samples from the clinic-based syndromic surveillance will also be tested using RT-PCR for Influenza A & B, HCoV NL63, OC43, HKU1, SARS-CoV & 229E as rule-outs, and SADS-CoV and HKU10 as an opportunistic survey for potential spillover these CoVs as proposed above.

2.7 Epidemiological analysis: We will conduct a case-control study to identify risk factors for SARSr-CoVs spillover. "Cases" are defined as participants whose samples tested positive for SARSr-CoVs by serological tests. "Controls" will be selected from the pool of participants admitted to the studies but testing negative. We will use nearest neighbor matching to pair cases demographically with controls at a 1-to-3 ratio or greater. We will use generalized linear models to analyze correlation between serological/PCR status and risk factors including: Activities with likely exposure to 1) bats; 2) livestock; and 3) locations of residence and work. We will use the same procedure to determine how clinical presentation differs between SARSr-COVs-exposed and unexposed enrollees, in the time course of illness, severity of symptoms, and type of symptoms.

2.8 Potential problems/alternative approaches: Rarity of spillover events means it may be difficult to identify sufficient seropositives to statistically analyze risk behavior or illness. First, we are now targeting our community-based surveillance to subpopulations with high-levels of bat exposure, at sites selected for diverse and prevalent SARSr-CoVs, and are adding clinic-based syndromic surveillance of SARI and ILI cases in these same regions – both will increase likelihood of finding positive individuals. Second, our serology testing will include a panel of assays for a large diversity of lineage 1 and 2 SARSr-CoVs as well as SADS-CoV, HKU10 and other bat-borne CoVs. *Rhinolophus* spp. bats host all of these (overall bat CoV PCR prevalence, 11.8%; β-CoV, 3.4%; α-CoV, 9.1%). Thus, using this broad serological panel to screen individuals in likely contact with these species increases the potential for detecting spillover with enough power for statistical analyses, and will shed light on behaviors that predispose to CoV spillover from bats. Third, we will include common human CoVs in our panel, so that even if low prevalence of bat CoVs is found, we will be able to conduct a valuable cross-sectional study of the seroprevalence of human CoVs. Finally, we will be able to assess relative measures of human-wildlife contact from our survey work. We will analyze intensity of contact against other risk factors and clinical outcomes to provide useful proxy information for spillover risk. **Patients visiting clinics may have cleared virus, but not yet developed IgG antibodies, reducing seropositive cases.** Our 35 day follow-up sampling should avoid this because the maximum lag time between SARS infection and IgG development was ~28 days (*53*). We also expect that patients in rural communities will only visit clinics when symptoms have progressed, likely coinciding with late illness and onset of IgG. We will also have data from our community study, so won't be completely reliant on hospital data to identify PCR- or seropositives. Finally, the risk is outweighed by the potential public health importance of discovering active spillover of a new SARSr-CoV. **Serological testing may not match known CoVs due to recombination events.** We will use the three-tiered serological testing system outline in **2.6.a** to try to identify these 'novel' CoVs, however, we will also remain flexible on interpretation of data to ensure we account for recombination.

Aim 3: *In vitro* and *in vivo* characterization of SARSr-CoV spillover risk, coupled with spatial and phylogenetic analyses to identify the regions and viruses of public health concern

3.1 Rationale/Innovation: In **Aim 1**, we aim to expand the known diversity of SARSr-CoVs by over 125 strains, targeting 10-25% S protein divergence that we predict infers high spillover risk and evasion of immune therapeutic and vaccine efficacy. In **Aim 3**, we will further characterize the zoonotic potential of a selected group of these novel SARSr-CoVs, using infectious clone technology, *in vitro* and *in vivo* infection experiments and analysis of HKU3r-CoV receptor binding to test the hypothesis that S protein % sequence divergence thresholds predict spillover potential (*18, 55*). We will analyze data from these viral characterization and infection experiments, coupled with bat host distribution, viral diversity and phylogeny, human survey of risk behaviors and illness, and human serology to assess spillover risk of SARSr-CoVs in different bat species across southern China. This will enable future development of public health interventions and enhanced surveillance to prevent the emergence of a novel SARSr-CoV.

3.2 General Approach: We will use S protein sequences to select a range of viral strains that cover the 10-25% S protein divergence we predict as high public health potential and construct chimeric SARSr-CoVs using the WIV1 backbone and these S genes as done previously (*12, 18, 38*). We will rescue of full-length clones and assess infection of non-permissive cells expressing human, bat and civet ACE2 receptors, Vero cells, primary human airway epithelial cells, and CaCo cells for HKU3r-CoVs (which have not been cultured and may use intestinal epithelium in nature). We will conduct experimental infections in hACE2 transgenic mice to assess pathogenicity and clinical signs (*18*). Finally, using a panel of mAbs that neutralize SARS-CoV infection *in vitro* and *in vivo*, and vaccine against SARS-CoV S protein, we will examine the capacity of strains with divergent S protein sequences to evade therapeutics, revealing strains with high public health potential. We will also conduct limited experiments to analyze HKU3r-CoV receptor binding and assess spillover potentia. Using these results, and data from Aims 1 and 2, we will use spatial modeling techniques to identify geographic hotspots in southern China where bat species that harbor high risk SARSr-CoVs inhabit, where communities that have high exposure to bats exist, where serological or PCR evidence of spillover has been identified, and where underlying demographic or environmental trends suggest high risk of future emergence.

3.3 Virus characterization: 3.3.a Construction of chimeric SARSr-CoV viruses: Infectious clones with the S gene of novel SARSr-CoVs and the SARSr-CoV WIV1 genome backbone using the reverse genetic system developed in our previous R01 (*24*). The correct infectious BAC clones will be screened by BAC DNA digestion with appropriate restriction enzyme or PCR amplification. The chimeric viruses will be rescued in Vero cells and then verified by sequence analyses. Our research group is well versed in coronavirus reverse genetics.

3.3.b Cell entry analysis: HeLa cells expressing human ACE2 are cultured on coverslips in 24-well plates incubated with the chimeric bat SARSr-CoVs with different spike proteins at a multiplicity of infection (MOI) = 1.0 for 1h. The inoculum is removed and the cells are washed twice with PBS and supplemented with medium. HeLa cells without ACE2 are used as negative control. Twenty-four hours after infection, cells are rinsed with PBS and fixed with 4% formaldehyde in PBS (pH7.4) at 4℃ for 20 min. ACE2 expression is detected by using goat anti-human ACE2 immunoglobulin followed by FITC-labelled donkey anti-goat immunoglobulin. Virus replication is detected by using rabbit antibody against the nucleocapsid protein of bat SARSr-CoV followed by Cy3-conjugated mouse anti-rabbit IgG. In parallel with the immunofluorescence assay, plaque assay will be conducted to determine the viral titers and growth kinetics in the infected cells at different times post-infection.

3.3.b Primary human airway epithelial cell culture: Primary human ciliated airway epithelial cells (HAE) cultures from the lungs of transplant recipients represent highly differentiated human airway epithelium containing ciliated and non-ciliated epithelial and goblet cells, grown on an air-liquid interface for several weeks prior to use (*18, 55, 56*). We will prepare HAE cultures from three different patient codes in triplicate in collaboration with the tissue procurement facility at the Cystic Fibrosis Center at UNC. Cultures will be inoculated with chimeric bat SARSr-CoVs to assess efficient replication. At 72 hpi, cultures will be fixed for immunofluorescent staining using antisera to the SARS-CoV conserved nucleocapsid protein (N) (*57, 58*). SARSr-CoVs that differ significantly in S protein sequence (11-24%) from epidemic SARS-CoV yet replicate *in vitro*, will also be evaluated for sensitivity to neutralization in Vero cells by PRNT50 assays using broadly SARS-CoV cross reactive human mAbs S227.14, S230.15, S215.17, and S109.8 (*49, 55*). As controls, the S

genes of novel SARSr-CoV will be inserted into VEE 3526 replicon vectors (VRP3526-S), packaged and used to vaccinate mice (*59*). Polyclonal sera will be harvested and tested for ability to cross neutralize SARS-CoV, GD03, WIV-1, SHC014, WIV-16, other novel SARSr-CoV and HKU3-SRBD by PRNT50 assay (*55, 60, 61*). Using PRNT50 titers with sera (n=4 each) among these viruses, antigenic cartography (*62*) will allow comparison of antigenic vs. phylogenetic distance, identifying the transition at which SARSr-CoV strains escape SARS-CoV based vaccines, informing immunotherapeutic and vaccine design strategies (*63-65*).

3.3.c Humanized mouse infection experiments: Briefly, in BSL3, n=5 10- to 20-week old hACE2 transgenic mice will be intranasally inoculated with 1 x 10^4 PFU of wildtype WIV-1 or chimeric bat SARSr-CoVs with different spike proteins, then monitored daily for weight loss, morbidity, and clinical signs of disease. Mice will be euthanized at 2, 4, and 6 dpi (endpoint of 14 dpi), organs harvested and virus quantified by SARS-CoV NP RT-PCR. After 7 days in formalin, tissues for histopathology will be removed from the BSL3 and stained with H&E, and for immunohistochemistry using polyclonal antibodies against the N protein. We will conduct limited evaluation of existing countermeasures using therapeutic monoclonal antibodies *in vitro* and *in vivo*. Existing SARSr-CoV mAbs will be diluted in DMEM starting at 1:20, and serial 2-fold dilutions mixed with an equal volume of 50 PFU of chimeric bat SARSr-CoVs with different spike proteins, then incubated on Vero E6 cells at 37°C for 1 h, then changed to a semi-solid medium (DMEM containing 1% methylcellulose and 2% FBS). Antibody neutralization titers will be evaluated by plaque quantification at 4-5dpi. hACE2 transgenic mice will be injected with SARS-CoV mAbs, and infected with chimeric bat SARSr-CoVs. Clinical signs and morbidity will be assessed and tissue pathology examined and compared with mice without treatment of mAbs to determine the therapeutic effect on SARSr-CoV infection, and protection of SARSr-CoV by wildtype SARS-S based vaccines assessed as described (*56, 66*). We will sequence full length genomes of high risk strains that are antigenically distinct and escape SARS cross neutralization, synthetically reconstruct a small subset (1-2) and evaluate the ability of nucleoside analogues to inhibit growth in HAE cultures and/or *in vivo* (*55, 56*).

3.3.d HKU3 clade cellular receptor: We will screen potential receptor molecules by pull-down analysis on membrane proteins interacting with the spike protein, initially using bat primary intestinal epithelial cell lines and lysates to extract protein, isolate membranes, and proteomically sequence intestinal proteins. The fusion protein of the HKU3 and HKU3r-CoV S proteins containing human Immunoglobulin Fc fragment will be eukaryotically expressed and purified. SARSr-CoV S will be incubated as bait protein with the membrane proteins extracted from *Rhinolophus sinicus* intestinal cells, to capture and precipitate membrane proteins that interact with the S protein. Mass Spectrometry will be performed to screen for the candidate receptor molecules and Co-Immunoprecipitation assay to confirm binding of the SARSr-CoV S protein to the candidate receptor. Alternatively, retroviruses pseudotyped with the SARSr-CoV S protein will be constructed and used to infect cells trans-expressing the candidate receptor molecule. Luciferase activity will be measured to test whether the S protein can bind to the receptor. If successful, this work will allow future research to clone and study human HKU3 receptor ortholog's ability to function as a receptor for other clade 2 strains and will allow better assessment of risk of clade 2 SARSr-CoV spillover to humans.

3.4 Combined spatial risk 'hotspot' analyses: We will use data from **3.3** to identify rank SARSr-CoV strains most likely to infect people and evade therapeutic and vaccine modalities. We will use bat survey and zoological data (*44*) to build species distribution models (*67*) and predict the distribution of bat species that harbor low, medium and high risk viral strains. Stacking these modeled distributions for the ~20-30 *Rhinolophus* and related species that occur in the region will allow estimates of SARSr-CoV diversity for a given locality. We will use machine learning models (boosted regression trees) and spatial 'hotspot' mapping approaches to identify the ecological, socio-economic and other correlates of SARSr-CoV diversity and spillover (from serosurveys) (*21, 68, 69*). We will include data from our human behavioral surveys and sampling to give a direct measure of where risk of spillover to people is likely to be highest in the region.

Potential problems/alternative approaches: We may not be able to glean further information about the **capacity of HKU3r-CoVs to infect human cells, or bind to human cell surface receptors.** If attempts at culture are unsuccessful, and efforts to identify the receptor too costly or time-consuming, we will cease this line of work. In that event, we will focus entirely on filling out the gaps in the 10-25% S protein sequence divergence from SARS-CoV, by working on a greater diversity of lineage 2 SARSr-CoVs.

Progress report publication list: R01 AI110964, Daszak PI, Project Period: 06/01/2014 – 05/31/2019

The following are peer-reviewed papers published from work funded by this NIAID R01 during the project period (*1-18*). Other manuscripts on behavioral risk, phylogenetic and viral risk characterization are in prep and awaiting submission prior to the end date of the grant.

1. B. Hu *et al.*, Detection of diverse novel astroviruses from small mammals in China. *Journal of General Virology* **95**, 2442-2449 (2014). PMID: 25034867
2. B. Hu, X. Y. Ge, L. F. Wang, Z. L. Shi, Bat origin of human coronaviruses. *Virology journal* **12**, (2015). PMID: 26689940
3. V. D. Menachery *et al.*, A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nature Medicine* **21**, 1508-+ (2015). PMID: 26552008
4. J. N. Mandl *et al.*, Reservoir Host Immune Responses to Emerging Zoonotic Viruses. *Cell* **160**, 20-35 (2015). PMID: 25533784
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9. X. L. Yang *et al.*, Isolation and Characterization of a Novel Bat Coronavirus Closely Related to the Direct Progenitor of Severe Acute Respiratory Syndrome Coronavirus. *Journal of Virology* **90**, 3253-3256 (2016). PMID: 26719272
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17. Z. Wu *et al.*, Comparative analysis of rodent and small mammal viromes to better understand the wildlife origin of emerging infectious diseases. *Microbiome* **6**, 178 (2018). PMID: 30285857
18. P. Zhou *et al.*, Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. *Nature* **556**, 255-258 (2018). PMID: 29618817

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	● Yes	❍ No
Is the Project Exempt from Federal regulations?	❍ Yes	● No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8	
Other Requested Information		

Human Subject Studies

Study#	Study Title	Clinical Trial?
1	Understanding the Risk of Bat Coronavirus Emergence: Community and clinic-based surveillance to capture SARSr-CoV spillover, routes of exposure and potential public health consequences	No

Section 1 - Basic Information (Study 1)

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

1.1. Study Title *

Understanding the Risk of Bat Coronavirus Emergence: Community and clinic-based surveillance to capture SARSr-CoV spillover, routes of exposure and potential public health consequences

1.2. Is this study exempt from Federal Regulations *	❍ Yes	● No

1.3. Exemption Number ❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8

1.4. Clinical Trial Questionnaire *

1.4.a. Does the study involve human participants?	● Yes	❍ No
1.4.b. Are the participants prospectively assigned to an intervention?	❍ Yes	● No
1.4.c. Is the study designed to evaluate the effect of the intervention on the participants?	❍ Yes	● No
1.4.d. Is the effect that will be evaluated a health-related biomedical or behavioral outcome?	❍ Yes	● No

1.5. Provide the ClinicalTrials.gov Identifier (e.g. NCT87654321) for this trial, if applicable

Section 2 - Study Population Characteristics (Study 1)

2.1. Conditions or Focus of Study

- Emerging zoonotic disease from bat Coronavirus

2.2. Eligibility Criteria

Participants to be enrolled in this study will be people living, working, or visiting the high-risk sites: 1) of likely divergence of SARSr-CoVs; 2) with a high diversity of SARSr-CoVs within the S protein sequence divergence of 5-25%; and 3) with high rates of human-wildlife interactions in four provinces of Yunnan, Guangxi, Guizhou, and Guangdong in China, who meet the inclusion criteria outlined below. Study sites are prioritized according to ecological and epidemiological conditions associated with a high risk for SARSr-CoVs spillover.

Research participants will be enrolled in two settings of community and hospital or clinic.

Community
People living, working, or visiting targeted high-risk communities (as defined above) who have close contact with bats, we anticipate interviewing and collecting biological samples from individuals with a range of exposure to bats. Enrolled research participants will be asked to provide biological samples and complete a questionnaire that is designed to obtain information about living circumstances (e.g. distance between the living house and closest bat roost, observed bats in house), income or livelihood, experience with SARI and ILI-like illness and involvement in activities with direct or indirect (e.g. via livestock) contact with bats.

Additional inclusion criteria for community participants
- Adults (18 years of age or older) who provide informed consent
- Pregnant women will be considered eligible for inclusion

Exclusion criteria for community participants
- Adults (18 years of age or older) who are unable to provide informed consent, including individuals with physiologically or medically induced cognitive impairments
- Individuals under 18 years of age
- Prisoners

Hospital or clinic
Patients at clinics or hospitals presenting with clinically defined symptoms of severe/acute respiratory illness (SARI/ARI) and/or influenza-like illness (ILI) with unknown origin. As with the community-based group, biological samples will be collected from the patients, and the patients or his/her designate will complete a questionnaire. We will follow up with these participants 35 days after enrollment to collect another biological sample to assess the development of IgG and collect additional data on the course of symptoms in the interim period.

Additional inclusion criteria for hospital or clinic participants
- Adults (18 years of age or greater) who provide informed consent
- Children aged 12 years or older with an accompanying parent or guardian who is able to provide informed consent, with the assent of children 12 years or older also required
- Pregnant women will be considered eligible for inclusion

Exclusion criteria for hospital or clinic participants
- Individuals over the age of 12 years who refuse to provide informed consent
- Adults unable to provide informed consent, including individuals with physiologically or medically induced cognitive impairments
- Children, aged 12-17, without an accompanying parent or guardian who is able to provide informed consent, or a child aged 12 to 17 who is unable or unwilling to provide assent
- Children < 12 years of age
- Prisoners

2.3. Age Limits	Min Age: 12 Years	Max Age: N/A (No limit)
2.4. Inclusion of Women, Minorities, and Children	NIAID_COV_2019_Inclusion_of_Women_Minorities_Children_Final.pdf	
2.5. Recruitment and Retention Plan	NIAID_COV_2019_Recruitment_Retention_Final.pdf	
2.6. Recruitment Status	Not yet recruiting	
2.7. Study Timeline	NIAID_COV_2019_Study_Timeline_Final.pdf	
2.8. Enrollment of First Subject	06/01/2020	Anticipated

INCLUSION OF WOMEN AND MINORITIES:

This proposal will enroll men and women as study participants without regard to ethnicity.

- At community sites, exposure to bats in working and living environments will be the primary criteria for identifying participants in community. We will make every effort to have men and women equally represented in this study and no individuals will be excluded based on ethnicity.

- At clinic sites, only patients who present at the health facility who meet the clinical case definition of 1) severe/acute respiratory illness (SARI/ARI) of unknown origin; or 2) Influenza-like illness (ILI) of unknown origin will be recruited for this study, and no patients will be excluded (or included) based on ethnicity or gender.

INCLUSION OF CHILDREN:

Children aged 12 years or older will be included in the clinical syndromic study.

- Previous clinic-based studies have shown that children are one of the major populations who are affected by the severe/acute respiratory illness (SARI/ARI) and/or Influenza-like illness (ILI).
- Children aged 12 years or older are post-primary school and are able to respond to the questionnaire on their own which increases the reliability of responses.
- Our human research team at the Institute of Pathogen Biology are all well-trained and have extensive experience working with children at this age, as well as their parents, in a clinical setting since 2009.
- Every effort will be made to protect the privacy, dignity, and well-being of children who participate in this study.
- Inclusion of children in the study would increase the sample size to allow for the estimation of effect sizes of behavioral risk factors by two-fold (2X) or greater with 80% power.

We will not include children in the community-based surveillance because children in target communities are mainly school children who have very limited exposures to bats or other wild animals under the scenarios of interest to the study, prolonged time spent in the forests or markets.

RECRUITMENT AND RETENTION PLAN

In order to improve recruitment within target communities, introductory visits will be made by project staff to each of the selected sites. These visits will be advertised through word of mouth and a project description letter to town/city leaders that can be posted in a central community location. The letter will inform the community that a team will be coming on a particular day(s) to discuss health issues related to animal contact. The letter will not be advertised for recruitment purposes. It will only be used to inform the community of the research visits. The project description letter will be written in the local language with a Flesch–Kincaid readability score equivalent to a 7th grade and up level (post-primary in China), to assure that potential community participants understand the study purpose, eligibility, and inclusion guidelines.

During community visits, discussions and meetings will be held firstly with local authorities and community leaders to introduce our project, and when appropriate and following approval from local authorities, the study team will post flyers to inform the community when the team will be coming back to speak about enrollment. This “town hall” style meeting will be completely voluntary, and, based on our experience, those interested would likely attend. Although local authorities may be present to introduce the study team members, they will not be involved in the recruitment and/or consent of the participants for the study. If research visits or recruitment are held at a workplace, subjects will be clearly informed during this recruitment process that their participation in the study is voluntary and will not impact their employment, nor will information discussed be shared with employers. With the local permission and accompanied will local authorities or community leaders, study team members will also engage in community ‘walkabouts’ during which they will discuss study details, as well as dates, times, and locations for enrollment and participation in the study.

Participation in the study will be strictly voluntary and will require signed informed consent for all participants and signed assent for clinical participants aged 12-17 along with parent or guardian consent. Participants will be given a consent form prior to being asked to participate in this study. Our research staff will read the consent form to potential participants, and they will review the consent form with the research staff and be given time to ask questions. After reviewing the consent form, study staff will explain details of the study including: why they were selected, what the study procedures are and what will be expected from them, potential risks and benefits of their participation, that their participation is completely voluntary, and that they can withdraw their participation at any time. Responses will be kept strictly confidential. Measures will be taken to assure the privacy, dignity, and respect of each participant. During training of research staff, we will emphasize the importance of avoiding coercion and protecting the privacy of participants.

Community-based recruitment: Participants from the community will be recruited through town hall meetings and community ‘walkabouts’ as described above. Meeting dates, times, and locations for enrollment and participation will be shared during these activities, and participants who wish to enroll can volunteer to participate at these times and locations.

Clinic-based recruitment: Patients eligible for enrollment will be identified at intake areas or in the emergency room, ward, or intensive care unit of each participating clinic and hospital by clinic staff, according to standard operating procedures at collaborating sites. Employed staff at each location will identify potential participants meeting the clinical case definition of severe/acute respiratory illness (SARI/ARI) and/or influenza-like illness (ILI) with unknown origin. Patients will be screened for eligibility according to the inclusion/exclusion criteria based on available clinical information. For larger provincial-level hospitals, interval sampling will be implemented by selecting every Nth case at the site among those individuals who meet enrollment criteria. The interval will be determined by local implementing partners based on an evaluation of the expected number of cases presenting at the site within a given year in order to best meet study design and sample size criteria. In terms of retention, we will express our gratitude to subjects for their participation and discuss the importance of the follow-up data collection. Nonetheless, we expect to have an approximate 40% loss to follow up and have included this in our sample size calculations.

STUDY TIMELINE

Patients/participants will be asked to volunteer approximately 1 hour of their time for participation in the study, including providing biological samples and completing the questionnaire at each sampling time point.

This will be an ongoing, five-year project (June 01, 2019 -- May 31, 2024). We anticipate to starting human subject enrollment on June 01, 2020, and completion of preliminary analyses is expected in 2024.

Inclusion Enrollment Reports

IER ID#	Enrollment Location Type	Enrollment Location
Study 1, IER 1	Foreign	Local community and hospital/clinic in Yunnan, Guangdong, Guangxi, Guizhou Provinces

Inclusion Enrollment Report 1

Using an Existing Dataset or Resource* : ❍ Yes ● No

Enrollment Location Type* : ❍ Domestic ● Foreign

Enrollment Country(ies): CHN: CHINA

Enrollment Location(s): Local community and hospital/clinic in Yunnan, Guangdong, Guangxi, Guizhou Provinces

Comments: This is a renewal, the cumulative enrollment from the previous funding period 5R01AI110964-05 is 980 females and 616 males, in total 1,596 Asians.
We don't plan to use the existing dataset.

Planned

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	4675	4675	0	0	9350
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	0	0	0	0	0
White	0	0	0	0	0
More than One Race	0	0	0	0	0
Total	4675	4675	0	0	9350

Cumulative (Actual)

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/ Alaska Native	0	0	0	0	0	0	0	0	0	0
Asian	0	0	0	0	0	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	0
Black or African American	0	0	0	0	0	0	0	0	0	0
White	0	0	0	0	0	0	0	0	0	0
More than One Race	0	0	0	0	0	0	0	0	0	0
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0	0	0	0	0

Section 3 - Protection and Monitoring Plans (Study 1)

3.1. Protection of Human Subjects — NIAID_COV_2019_Protection_Human_Subjects_Final.pdf

3.2. Is this a multi-site study that will use the same protocol to conduct non-exempt human subjects research at more than one domestic site? — ❍ Yes ● No ❍ N/A

If yes, describe the single IRB plan

3.3. Data and Safety Monitoring Plan

3.4. Will a Data and Safety Monitoring Board be appointed for this study? — ❍ Yes ● No

3.5. Overall structure of the study team

PROTECTION OF HUMAN SUBJECTS:

1. Risks to Human Subjects

1.1 Human Subjects Involvement, Characteristics, and Design

This project is a study of human exposure to animal coronaviruses in southern China. Subjects will be enrolled on a voluntary basis and informed consent will be obtained from all participants. Consenting participants will provide biological samples and complete a questionnaire. Subjects will be individuals: 1) who are highly exposed to bats in community settings, including through hunting, butchering, or general handling within the context of their living or working environment (≥ 18 years old); and 2) patients admitted to hospitals and clinics presenting with disease symptoms of clinically-defined severe/acute respiratory illness (SARI/ARI) or Influenza-like illness (ILI) of unknown origin (≥ 12 years old).

The study population will be selected from the Yunnan, Guangxi, Guangdong, and Guizhou provinces of China. We will aim to enroll: 1) in 12 clinic sites across the four provinces, 2,750 individuals (accounting for an estimated 40% loss from follow-up); and 2) in 8 community sites, 1,650 individuals per each of the four provinces, pooled across two sites for each province for a total of 6,660 (1,650*4) participants, allowing us to make province-level comparisons of differing effects (one time data collection, no follow-up among community participants). The community and clinical sites are further defined in **"Specific Aim 2: Using community-based and clinical biological-behavioral surveys to identify SARSr-CoV spillover, routes of exposure and public health consequences of human infection"**.

There are no data to suggest a gender or ethnic bias for coronavirus exposure or infection, therefore subjects will be enrolled based on exposure criteria, and subjects will not be excluded based on ethnicity or gender. We will also stratify sampling to ensure representation of sex, demographic, and socio-economic factors in each community site.

1.2 Sources of Materials

Samples to be collected and screened for coronaviruses include whole blood and nasal/oropharyngeal swabs. Samples will be collected and a questionnaire will be administered by trained medical personnel from the local CDC, hospitals, and clinics. In community sites, whole blood samples (only) will be collected once during Years 2-4 of the study, and samples will be screened for coronaviruses using developed ELISA at the Institute of Pathogen Biology and the Wuhan Institute of Virology. In clinic sites, both whole blood samples and nasal/oropharyngeal swabs will be collected at enrollment, and samples will be screened for coronaviruses using ELISA and consensus PCR (cPCR). Patients who test positive for coronavirus or antibodies to coronavirus will be followed up 35 days after enrollment, when additional blood samples will be collected for serological testing with ELISA.

At the enrollment, a standardized questionnaire will be administered at both community and clinic sites to collect data on living circumstances (e.g. distance between the living house and closest bat roost, observed bats in house), income or livelihood, experience with SARI and ILI-like illness and involvement in activities with direct or indirect (e.g. via livestock) contact with bats. During the follow-up with clinic study participants, a standardized questionnaire supplement will be administered to collect additional data on the course of symptoms in the interim period. All electronic data will be password protected, and all hardcopy files and biological samples will be stored in secure storage facilities. All consent forms will be stored separately from any data in separated locked filing cabinets.

1.3 Potential Risks

The potential risks to study participants resulting from study participation are minimal. The volume of blood being collected is within normal safety limits. The questionnaire will be designed to assess exposure risk, and may ask personal questions, but they will conducted in private and confidentially to protect privacy. There may be some stress to subjects who are informed that they have been exposed to an animal virus, but counseling will be available and options for medical care will be included in the discussion.

2. Adequacy of Protection against Risks

2.1 Recruitment and Informed Consent

Potential study participants at each site will be identified by well-trained in-country research team in partnership with local CDC staff (for community participants) and medical personnel (for clinic participants). The team will be thoroughly trained on communicating the research objectives, what is being asked of participants, any risks or benefits, and will be able to address any questions that potential subjects may have. Both written and oral descriptions of the study details will be provided in Chinese Mandarin (or orally via an interpreter in local dialects if necessary) as part of the informed consent process. Contact details of the collaborators at the local CDC or hospital and the study PI will be provided to all subjects, and CDC or hospital personnel on the research team will be available onsite to answer questions from the study subjects. Test results will be communicated to each subject and counseling offered to minimize stress.

2.2 Protection against Risks

After the informed consent process, the questionnaire will be conducted in private, ensuring that others cannot overhear responses. Individual sessions will be held in areas where there are no other individuals within a 10-foot distance. A barrier will be created so that no other individuals can view the participants during their interview. Depending on the location, this could be a private room, behind a building or fence, or behind a line of trees, obstructing view so that confidentiality may be maintained. The interview team will take care to pair interviewers and respondents by sex to the best of their ability to increase the level of comfort of the participant and the team will ensure the privacy and confidentiality of responses. Children will not be interviewed in the absence of a parent or guardian. This study will not involve greater than minimal risk, and every effort will be made to ensure the privacy, dignity, and well-being of children who participate in this study.

3. Potential Benefits to Subjects and Others

There are potential benefits to the study subjects including receiving a physical exam/health check from a medical officer and the potential benefit of identifying a health hazard. At the conclusion of the study, we will deliver an educational workshop for high risk individuals (open to study subjects and non-study subjects) describing the health benefits of using PPE and hand-washing during animal handling activities throughout the day, as well as to share other prevention interventions that emerge from the research data.

4. The Importance of Knowledge to be Gained

There are valuable potential benefits to the general public from the knowledge to be gained by this study, as it may identify sources of zoonotic coronaviruses in the market system or through hunting. Avoidance of these animals or extra care when handling them may substantially reduce the risk of CoVs (and other zoonotic pathogen) transmission.

Section 4 - Protocol Synopsis (Study 1)

4.1. Brief Summary

4.2. Study Design

4.2.a. Narrative Study Description

4.2.b. Primary Purpose

4.2.c. Interventions

Type	Name	Description

4.2.d. Study Phase

Is this an NIH-defined Phase III Clinical Trial? ❍ Yes ❍ No

4.2.e. Intervention Model

4.2.f. Masking ❍ Yes ❍ No

❑ Participant ❑ Care Provider ❑ Investigator ❑ Outcomes Assessor

4.2.g. Allocation

4.3. Outcome Measures

Type	Name	Time Frame	Brief Description

4.4. Statistical Design and Power

4.5. Subject Participation Duration

4.6. Will the study use an FDA-regulated intervention? ❍ Yes ❍ No

4.6.a. If yes, describe the availability of Investigational Product (IP) and Investigational New Drug (IND)/ Investigational Device Exemption (IDE) status

4.7. Dissemination Plan

Delayed Onset Studies

Delayed Onset Study#	Study Title	Anticipated Clinical Trial?	Justification
The form does not have any delayed onset studies			

VERTEBRATE ANIMALS:

1. Detailed description of animal use.

Work with vertebrate animals will be conducted at Wuhan University at the School of Medicine in Wuhan, China and the University of North Carolina, Chapel Hill, USA.

Capture and sampling techniques for all wild animals (bats) described in this study have been previously approved by multiple Institutional Animal Care and Use Committees (IACUCs) for projects led by EcoHealth Alliance. These institutions include: UC Davis IACUC (Mazet and Epstein; UC Davis 15898; current); and The Cummings School of Veterinary Medicine at Tufts University (Olival and Epstein, current), Animal Welfare Assurance (#A4059-01) on file with the Office of Laboratory Animal Welfare at the National Institutes of Health. We have prepared a draft IACUC application for this project, and will submit it within 1 month of the project's start date (to Tufts University) to minimize delays in beginning Year 1 field sampling.

Experimental work using humanized mice will be conducted at the Center for Animal Experiment Biosafety 3 lab of Wuhan University at the School of Medicine in Wuhan, China and the University of North Carolina at Chapel Hill, the Institute for Pathogen Biology. The Wuhan laboratory is AAALAC accredited and has both an Institutional Biosafety Committee and an Institutional Animal Care and Use Committee. We will submit our protocols for IACUC approval should this proposal be funded. Conditions for animal use are described below. Both laboratories (Wuhan and UNC) have Internal Biosafety Committees and are accredited BSL-2 and BSL 3 laboratories. Animals will be housed in a BSL-3 facility and will be under the care of a full-time veterinarian. All experimental work using infectious material will be conducted under appropriate biosafety standards. Disposal of hazardous materials will be conducted according to the institutional biosafety regulations.

Note: The majority of wild bats captured and sampled will be done using non-destructive, techniques. In a small number of instances (~ 2 bats per species for previously unsampled *Rhinolophus* sp.), where intestine and lung tissue is required to establish cell lines, animals will be humanely euthanized and a necropsy performed according to accepted protocols (see euthanasia section)

Bat capture. Free-ranging bats will be captured using either a mist net or harp trap. The net system is manned by two people during the entire capture period, and bats are removed from the net as soon as they become entangled to minimize stress and prevent injury. In the Co-PI's (Dr. Olival) experience, a maximum of 20-30 bats can be safely held and processed by a team of three people per trapping period. Duration of trapping will depend on the capture rate. Bats are placed into a pillowcase or small cloth bag and hung from a branch or post until samples are collected. Bats are held for a maximum of six hours (typically less than 3 hours), and released after sampling.

Laboratory mice. Lab mice will be sourced commercially by the Wuhan Center for Animal Experiment at Wuhan University.

Sample Collection:

Bats: Bats will be manually restrained during sampling. Depending on the species and size of bat, swabs will be taken from the oropharynx, urogenital tract, and rectum. Fresh feces will be collected if available, in which case a rectal swab will not be collected. Blood will be collected from fruit bats either from the cephalic vein or from the radial artery or vein using a 25 gauge needle and 1cc syringe. Blood

will be collected from bats weighing less than 100g according to published techniques (*126*).
Rodents: Anesthesia for captive small rodents will be conducted using plastic tubes, with the animals transferred directly from the traps to the tubes containing a cotton swab soaked in ether, isoflurane, or methoxyflurane for anesthetic induction. For larger rodents, chemical restraint and anesthesia (ketamine alone, or ketamine combined with xylazine) will be applied either through the squeeze cages by syringe if applicable. Once anesthetized a small blood sample will be collected using a capillary tube placed into the retro-orbital sinus. Only trained technicians will perform retro-orbital bleeding and it will only be performed on anesthetized rodents. Femoral or jugular venipuncture may be used for larger rodents (e.g. rats). In all rodents, blood volumes of no more than 1% of body weight will be withdrawn. (example 0.2 ml blood from a 20 gram rodent).

Laboratory Mice. Humanized mice will be bred at the University of Wuhan and University of North Carolina at Chapel Hill. Mice will be inoculated with a specific dose (e.g. $1x10^6$ TCID50) of virus through different routes (intranasally and intraperitoneally). Mouse body temperature will be monitored with implanted temperature sensing microchips (LifeChip Bio-thermo, Destron Fearing), and mice will be weighed daily. Animals will be observed daily for clinical signs of illness. Moribund mice will be euthanized, according to AVMA recommendations. Live animals will be euthanized at three weeks post-inoculation and organs harvested. We will collect sera on days 10, 15 and 21 to test for neutralizing antibodies against bat CoVs. We will collect nasal washes, oral swabs, and rectal swabs, and urine every two days. These are minimally invasive procedures, and will be performed by experienced lab technicians under the supervision of a full-time veterinarian.

2. **Justify use of animals, choice of species, numbers to be used.** Species and number used in study: The purpose of this study is to conduct targeted, but extensive surveillance of bat populations in Southern China to detect coronaviruses that may pose a risk to the health of both humans and animals. The experimental work is designed to understand the ability of bat coronaviruses to bind to human receptors. In this renewal application, we propose a total bat sample size comparable to our initial R01 effort of ~5000 animals. These animals will be sampled from ~15-20 species collected across 4 provinces. Given ~5-12% prevalence of SARSr-CoVs in *Rhinolophus* spp. at our previous sites during our initial R01, this sample size would give us 425 (±175) positive individual bats, and allow us to identify ~125 novel strains. Assuming a conservative prevalence rate, a sample size of n=110 individuals per species will allow us to detect SARSr-CoV using PCR with a power of 80%. **Wild bats**: We will sample a minimum of 110 individuals from ~15-20 different bat species from sites across four provinces in Southern China (Yunnan, Guangxi, Guizhou and Guangdong). Sampling will focus on species in the family *Rhinolophidae,* genus *Rhinolophus.,* but will also include individuals in the related genera *Hipposideros* and *Aselliscus.* In every situation, sampling of wildlife will be conducted in the most humane manner while minimizing the impacts on individual animals and their wild populations. In all instances, the fewest number of animals will be sampled that will provide valid information and statistical inference for the pathogen and disease of interest and every effort will be made to minimize stress and discomfort for the animal.

A small number of bats (maximum 2 per species) representing each of the species in this study may be euthanized in order to collect lung and intestinal tissue required for characterizing coronavirus receptors. Voucher specimens may also be collected at the discretion of the team leader for the accurate identification of species using molecular methodology.

Humanized mice for experimental infection for Specific Aim 3: In order to understand whether select strains of bat-borne CoVs utilize receptors found in people have the potential to infect people, we will use Swiss albino mice (standard breed at Wuhan University) that have been genetically modified to have

human receptors. We'll infect them with cultured bat coronaviruses and determine which organs become infected and whether these mice are capable of shedding infectious virus. Humanized mice will be genetically modified to carry human ACE2 gene will be used to evaluate pathogenesis of CoVs. We cannot anticipate exactly how many viruses we will find that are candidates for experimental models, however will likely identify approximately 5-6 bat SARSr-CoV strains that will be used for mouse infection experiments. We will use 15-20 adult mice per virus strain, and therefore will require a maximum of 120 mice over the study period.

3. **Provide information on veterinary care.** For wild caught animals, **t**here is no specific veterinary care that is appropriate, nor will clinical veterinary facilities be available. Animals that are injured during the capture or sampling process will be assessed by an experienced team leader, and if the animal is determined to be unlikely to survive if released, it shall be euthanized humanely (see euthanasia section). Animals will be released within hours of capture.

Laboratory mice will be housed in BSL-3 small animal facilities at the Center for Animal Experiment at Wuhan University and University of North Carolina at Chapel Hill, the Institute for Pathogen Biology. Experimental animals will be regularly monitored by experienced staff and a supervising veterinarian. The animal facility operates 24 hours a day and has full-time veterinarians on staff. All animals will be provided with food and water ad libitum and will otherwise receive standard care.

4. **Procedures for ensuring animal comfort, lack of distress, pain, or injury:** Wild bats will not be held longer than 6 hours during the sampling process. Co-PI Olival has extensive experience in capture, anesthesia, and sampling wildlife, especially bats. In our team's experience, bats tolerate the described procedure well. Mist nets will be attended continuously during capture periods, and bats will be extracted from the net as soon as they become entangled. This will minimize stress and injury from entanglement. Bats will be placed individually in cotton bags and hung from tree branches while awaiting processing and during recovery. The bags are sufficiently porous as to allow for ventilation and are designed for bat capture. The enclosed environment seems to calm the bats, as they do not struggle once inside, but they hang quietly – this is a standard and accepted practice in the bat research world and best way to minimize stress to the animal. Animals will be monitored by a veterinarian or experienced field team member during all stages of capture, processing, and release. Animals will be kept in a cool place while in the pillowcases.

The procedures used in this experiment (blood draw, nasal, oral, and rectal swabs) are minimally invasive. Mice that show signs of morbidity post-infection will be examined and euthanized according to AVMA standards (see below).

5. **Euthanasia:** In the event of injury to an animal that results in pain and suffering, and reasonable veterinary care is unavailable, the animal will be euthanized by a veterinarian or trained field team member using ketamine injected intramuscularly 37.5mg/kg and sodium pentobarbital injected intravenously at a dose of 1.0ml per 5kg injected intravenously. This protocol is in accordance with the AVMA euthanasia report (2007). Any animal that is euthanized using a chemical agent will be disposed such that it will not be permitted to enter the food supply either through markets or hunting.

SELECT AGENT RESEARCH/BIOHAZARDS. No select agent research.

Agents: SARS-related bat coronaviruses (SARSr-CoV), like WIV1, WIV16 and SHC014. These bat viruses are distantly related to the epidemic human SARS-CoV which emerged in 2003 and caused 8,000 cases and 800 deaths worldwide. While the epidemic human SARS-CoV is a BSL3 select agent, the SARSr-CoV are BSL3 pathogens in the US and not select agents. The proposal will use a SARSr-CoV molecule clone designated WIV1 during the course of these studies, which is NOT a select agent. This strain has not been shown to cause human disease or be transmissible between humans. All recombinant DNA work will use the bat SARSr-CoV WIV1 molecular clone. At the University of North Carolina (US Government select agent certified laboratory), some virus growth studies will be conducted in primary human airways, comparing wildtype SARS-CoV, WIV1 and various SARSr-CoV WIV1 chimeric virus growth kinetics. Wildtype SARS-CoV strain research will not be conducted at the Wuhan Institute of Virology.

Registration status of all entities where select agent(s) will be used. Wildtype SARS-CoV is a select agent. UNC-Chapel Hill is currently registered with the CDC for select agent use, including SARS-CoV, as required by select agent regulations (42 CFR 73). The UNC SARS select agent laboratories are routinely inspected by the environmental health and safety department at UNC and by the CDC. Workers receive select agent and BSL3 training focused on SARS-CoV safety, procedures and protective clothing/PAPR training each year.

Introduction and Background. SARS-CoV caused outbreaks with significant case fatality rates, and there are no vaccines available for this agent. SARS-CoV is classified as a BSL-3 select agent. Wildtype SARS-CoV is currently thought extinct in the wild. The work proposed in this application will involve two aspects: field work and laboratory work, focusing on distantly SARS-like bat coronaviruses (SARSr-CoV). Fieldwork involves the highest risk of exposure to SARSr-related or other bat CoVs, while working in caves with high bat density overhead and the potential for fecal dust to be inhaled. There is also some risk of exposure to pathogens or physical injury while handling bats, civets, rodents or other animals, their blood samples or their excreta. The Co-PIs and field team have extensive experience and certification working with wildlife species and high-biosecurity pathogens (Nipah virus, ebolavirus, SARS), and great care will be taken in the field to limit the risk of accidental exposure to known or unknown animal pathogens. We have strict procedures for handling bats and working with samples from them as they are secured in the field and transported to the lab. Field team members handling animals will be trained to utilize personal protective equipment (PPE) and practice proper environmental disinfection and biosafety techniques. This includes wearing coveralls or dedicated clothing, nitrile gloves, eye protection, and a P95 or P100 respirator during bat handling and sampling. Fully Tyvek suits and HEPA-filtered Powered Air Purifying and Supplied Air Respirator Systems (PAPRs) will additionally be worn in cave systems where there is a higher risk of contact with aerosolized bat feces. All field clothing and equipment will be disinfected using Virkon disinfectant. All biological waste from field surveys will be disposed of in the appropriate container (sharps box or an autoclave bag) and will be autoclaved at local hospitals or university labs. All personnel will be vaccinated against rabies and have a neutralizing antibody titer, in accordance with WHO and CDC recommendations. Field teams will carry rabies boosters in the field and will receive a booster in the event of a potential rabies exposure.

Field safety protocol: Our procedures to deal with bites, needle-sticks etc. are as follows: The wound is washed thoroughly with soap and water to clean away dirt and debris, then vigorously scrubbed with a sterile gauze bandage and benzalkonium chloride for 5 minutes. If bleeding, pressure is applied with a sterile bandage for until bleeding has stopped. If the wound continues to bleed, medical attention at the nearest hospital is sought. The bat from which the bite or exposure originated is identified, and the samples collected from it labeled on the data sheet that these were involved in an exposure. Our procedures require that the person potentially exposed reports to a major hospital within 24 hours to

have wound examined and receive a rabies post-exposure booster vaccines as per WHO/CDC protocols. The laboratory work is lower risk, as samples placed in lysis buffer will be non-infectious. Samples placed in viral transport medium and frozen will be stored at ultra-low temperatures (-86˚C) until viral isolation is required. Serum will be heat inactivated at 56˚C for 30 minutes prior to testing.

Lab biosafety: The University of North Carolina at Chapel Hill, the Institute for Pathogen Biology, the Wuhan Institute of Virology, and the Wuhan University Center for Animal Experiment BSL-3 laboratories all have respective Internal Biosafety Committees and are accredited BSL-2 and BSL 3 laboratories. All experimental work using infectious material will be conducted under appropriate biosafety standards. Disposal of hazardous materials will be conducted according to the institutional biosafety regulations.

Available Treatments: No approved treatments are related for SARS and the SARSr-related bat coronavirus infections. However, therapeutic antibodies and nucleoside analogues have been successfully used in SARS-infected rodents and primates, which could be approved for compassionate use in humans exposed to the SARSr-CoV.

UNC Facilities where the select agent(s) will be used. SARS-CoV will be manipulated in research activities including establishment of viral replication curves, infection of rodent animal models and performance of plaque assays in laboratory spaces that meet operational and procedural criteria for BSL-3 activities as outlined in the CDC/NIH “Biosafety in Microbiological and Biomedical Laboratories”, 5th edition, as well as BSL-3 criteria outlined in the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (April 2016). In addition, all mouse studies at UNC-Chapel Hill will be performed in an approved and registered BSL-3/ABSL-3 laboratory equipped with Techniplast Sealsafe^{TM} HEPA-filtered animal housing for rodents. All animal protocols will be approved by the UNC-Chapel Hill IACUC.

UNC BSL-3/ABSL-3/select agent laboratories are equipped with biosafety cabinets, incubators, centrifuges with containment features, cold storage units, an autoclave, sink, eyewash and life safety equipment, and mechanical system monitors and alarms to support effective isolation and containment of operations involving SARS-CoV and SARSr-CoV. The anterooms to the BSL-3 laboratories house PAPR charging stations, laboratory and safety supplies, and a changing area. For both the BSL-2 and BSL-3 select agent spaces, access to select agents is restricted by the door between the hallway and anteroom and the door between the anteroom and BSL-3 space, requiring a combination of swipe card and punch code for entry. All select agent materials (SARS-CoV virus and genome length RNA) are stored in locked freezers and incubators.

UNC Procedures for monitoring possession, use and transfer of select agents. All personnel who will have access to select agent-regulated materials have been added to the Select Agent registration following security risk assessments prescribed by the CDC Select Agent Program. Personnel have completed training in all aspects of select agent compliance requirements and have adopted changes to standard operating procedures as applicable to assure that these requirements are met. Personnel will follow all procedures prescribed for accessing and securing the laboratory, documenting laboratory activities and materials used, and responding to incidents that could result in theft, loss, or release of select agent-regulated materials. Transfers of select agent-regulated materials will be coordinated by the laboratory managers and Responsible Official in accordance with standard operating procedures, including obtaining appropriate permits for shipping select agent materials and observing all regulations for shipping, both under dangerous goods and select agent regulations. Transfer of select agent RNA in TRIzol from registered BSL-3 to registered BSL-2 space and cDNA from registered BSL-2 space to non-registered BSL-2 space is conducted according to current select agent rules, regulations, and guidelines, including the new inactivation policies released in 2017.

UNC Biosafety, biocontainment, and security of the select agent(s). The Baric laboratories have been operational with BSL-3 core policies and procedures for ~15 years. Standard operating procedures at BSL-3 have been reviewed and approved by the UNC Chapel Hill Institutional Biosafety Committee and undergo both annual review and approval as well as updates as laboratory processes change or biosafety procedures evolve. The content of these documents has been formatted to conform to select agent regulations for the biosafety, security, and incident response plans. Additionally, lab-specific security risk assessments have been completed and recommendations implemented to ensure that security measures and procedures are sufficient to effectively minimize the possibility of unauthorized access to select agent-regulated materials. The UNC Chapel Hill facilities have undergone multiple CDC inspections and are currently in compliance with CDC requirements relating to SARS-CoV and select agent status. Our three-year renewal inspection occurred in June 2018 and we have been renewed for another three years.

UNC Biocontainment resources. All BSL-3 laboratories are under negative pressure, with redundant systems to ensure that negative pressure is maintained. All BSL-3 facilities have autoclaves to decontaminate waste materials as well as approved protocols for treatment or inactivation of any materials leaving the laboratory. All personnel are extensively trained in basic virology and safety protocols before being approved for select agent work and undergo additional extensive training to work with SARS-CoV and related SARSr-CoV as a BSL-3 pathogen. In both laboratories, annual testing is performed to verify that biosafety cabinets, laboratory supply/exhaust systems (including alarms), and other laboratory equipment are functioning as designed. The laboratories are secured at all times, and only personnel who have successfully completed Select Agent clearance and laboratory specific training requirements are permitted to enter without an escort.

P3CO Research. Recognizing the implementation of new gain of function research guidelines under P3CO, SARS-CoV and MERS-CoV are subject to these guidelines, and as such, reverse genetic studies are subject to review. Our group has considerable expertise in interfacing with the appropriate NIH P3CO institutional review boards to review, revise and finalize research designs that have the potential to modify pathogenesis or transmissibility in mammals. **Importantly, we are not proposing to genetically manipulate SARS-CoV** over the course of this proposal. However, we are proposing to genetically manipulate the full length bat SARSr-CoV WIV1 strain molecular clone during the course of the proposal, which is not a select agent, has not been shown to cause human infections, and has not been shown to be transmissible between humans.

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CONSORTIUM/CONTRACTUAL ARRANGEMENTS:

This project is a multi-institutional collaboration led by EcoHealth Alliance, New York (Daszak, PI), which will subcontract funds to three institutions: the Wuhan Institute of Virology (Dr. Shi), the University of North Carolina at Chapel Hill (Dr. Baric), and the Institute of Pathogen Biology (Dr. Ren). In addition, Dr. Linfa Wang from Duke-National University of Singapore (Duke NUS) will act as a senior consultant with no requested subcontract funds, and will primarily advise on the serological and molecular based diagnostic platforms. Dr. Daszak has over 15 years previous experience managing collaborative projects including two R01s on Nipah virus ecology and the current R01 on Coronavirus (AI110964) that involve multiple, separate foreign institutions; a 5-year NSF/NIH Ecology of Infectious Disease award on West Nile virus which involved multiple subcontracts, a NIAID R01 on bat viral discovery that involved multiple international contracts, and a multi-million dollar per year contract from USAID that involves 21 international partners. The applicant organization (EcoHealth Alliance) is justified in taking the lead on this project because it specializes in understanding the ecological, and virological processes underlying zoonotic disease emergence. Dr. Daszak has conducted significant preliminary work on this issue including 15-years of research on the ecological and related factors of the emergence of SARS and 15-years of work in China. The subcontract institutions will work on specific issues and areas in which they have proven expertise. These areas are:

- Human community surveillance, human clinical or hospital syndromic surveillance, CoV serology, full genome sequencing, epidemiology, and behavioral risk (Institute of Pathogen Biology, Dr. Ren)
- CoV screening and serology of non-human samples, viral pathogenesis, serological testing protocol development, host receptor binding, S protein sequencing, *in vitro* and *in vivo* virus characterization (Wuhan Institute of Virology, Dr. Shi)
- Small animal models of viral pathogenesis, primary human cell cultures, CoV reverse genetics, reconstruction of zoonotic CoV (University of North Carolina at Chapel Hill, Dr. Baric).

Dr. Daszak has had inter-institutional contractual agreements with the Wuhan Institute of Virology for over 13 years. Drs. Shi and Daszak have collaborated together since 2002 and have been involved in running joint conferences, collaborating on papers, and shipping samples into and out of China. Drs. Baric, Shi, Wang, and Daszak have collaborated closely for over 10 years on Coronavirus and other emerging disease research. Drs. Shi and Ren have collaborated on viral discovery projects 8 years.

WUHAN INSTITUTE OF VIROLOGY
THE CHINESE ACADEMY OF SCIENCES

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31 October 2018

Dr. Peter Daszak
President
EcoHealth Alliance
460 W 34th St. 17th Floor
New York, NY 10001 USA

Dear Dr. Daszak,

The Wuhan Institute of Virology, Chinese Academy of Sciences, has an high interest in working with EcoHealth Alliance and its scientists to identify and prevent the transmission of bat coronaviruses to human populations globally. In particular, the NIAID funded R01 renewal proposal entitled "Understanding the risk of bat coronavirus emergence" will provide an excellent opportunity to achieve these goals.

The Wuhan Institute of Virology, Chinese Academy of Sciences, recognizes the mutual benefits to be gained through research cooperation and a successful partnership with EcoHealth Alliance in the field of identification and prevention of zoonotic disease transmission. It is vital to not only identify the diseases themselves, but also identify high-risk human populations and the actions that put them at risk for infection along with evaluating approaches to intervention and disease management.

Understanding and preventing exposure and transmission of zoonotic diseases from wildlife to humans remains a high priority for prevention of pandemics. In our discussion with EcoHealth Alliance, we have agreed to participate in activities that will strengthen the ability of China and other countries in the region to respond to epidemic disease outbreaks – particularly those of animal origin. To assist in this study, we will provide participating laboratories in China with human samples both new and archived and support research in bat coronaviruses.

We at Wuhan Institute of Virology, Chinese Academy of Sciences, look forward to our continued collaborations with the EcoHealth Alliance team and working further on this worthwhile study.

Sincerely,

Dr. Yanyi Wang
Director, Wuhan Institute of Virology
Chinese Academy of Sciences
Xiao Hong Shan, No.44
Wuhan 430071 China
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[redacted]

中国医学科学院
北京协和医学院 病原生物学研究所

01 November 2018

Dr. Peter Daszak
President
EcoHealth Alliance
460 W 34th St. 17th Floor
New York, NY 10001 USA

Dear Dr. Daszak,

The Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College (IPB, CAMS&PUMC) has an high interest in working with EcoHealth Alliance and its scientists to identify and prevent the transmission of bat coronaviruses to human populations globally. In particular, the NIAID funded R01 proposal entitled "Understanding the risk of bat coronavirus emergence" will provide an excellent opportunity to achieve these goals.

The IPB, CAMS&PUMC recognizes the mutual benefits to be gained through research cooperation and a successful partnership with EcoHealth Alliance and long term colleague Dr. Zhang Shu-Yi in the field of identification and prevention of zoonotic disease transmission. It is vital to not only identify the diseases themselves, but also identify high-risk human populations and the actions that put them at risk for infection along with evaluating approaches to intervention and disease management.

Understanding and preventing exposure and transmission of zoonotic diseases from wildlife to humans remains a high priority for prevention of pandemics. In our discussion with EcoHealth Alliance, we have agreed to participate in activities that will strengthen the ability of China and other countries in the region to respond to epidemic disease outbreaks – particularly those of animal origin. To assist in this study, we will provide participating laboratories in China with human samples both new and archived and support research in bat coronaviruses.

We at IPB, CAMS&PUMC look forward to our continued collaborations with the EcoHealth Alliance team and working further on this worthwhile study.

Sincerely,

Lili Ren
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October 24, 2018

Peter Daszak, PhD
EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

Reference: *Program Announcement number PA-18-484, entitled* NIH Research Project Grant (Parent R01 Clinical Trial Not Allowed), *dated December 6, 2017*

Dear Dr. Daszak,

This letter confirms that the appropriate program and administrative personnel at The University of North Carolina at Chapel Hill (UNC-CH) have reviewed the above referenced program announcement and are committed to enter into a subcontract with the EcoHealth Alliance for the performance period of July 1, 2019 to June 30, 2024. The work to be performed by UNC-CH does not include animal and/or human research subjects. UNC-CH maintains an active and enforced conflict of interest policy meeting the requirements of 42 CFR Part 50, Subpart F and 45CFR Part 94.

EcoHealth Alliance's Principal Investigator on this proposal is Dr. Peter Daszak. The UNC-CH budget, budget justification and scope of work are provided as separate enclosures to this letter. The estimated cost of the proposed subcontract will not exceed $388,750 and includes appropriate direct and indirect costs.

Furthermore, by submission of this commitment letter UNC-CH and its Principal Investigator (PI) certify (1) that the information submitted within the application is true, complete and accurate to the best of the UNC-CH's and PI's knowledge; (2) that any false, fictitious, or fraudulent statements or claims may subject the UNC-CH and PI to criminal, civil, or administrative penalties; and (3) that the PI agrees to accept responsibility for the scientific conduct of the project and to provide the required progress reports if an award is made as a result of UNC-CH's application.

If you have any questions, please contact the undersigned at 919-966-3895.

Sincerely,

Terry Magnuson, PhD
Vice Chancellor for Research

Ralph S Baric, PhD
Professor, Epidemiology, Microbiology and Immunology

Enclosed:
Budget
Budget Justification
Scope of Work

DukeNUS Medical School

31 Oct, 2018

Dr. Peter Daszak
EcoHealth Alliance
460 West 34th Street, Suite 1701
New York, NY 10001, **USA**

Dear Peter,

I am writing this letter in strong support of the proposed renewal of the NIH (R01AI110964, Understanding the Risk of Bat Coronavirus Emergence) project led by EcoHealth Alliance.

As you know, I have long experience with EIDs associated with bats, having worked with Hendra virus in Australia, Nipah virus in Malaysia, Singapore and Bangladesh, SARS related viruses in China, Reston ebolavirus in the Philippines. More recently, in collaboration with scientists in EcoHealth and China, I played an important leadership role in coordinating and directing the research which discovered abat HUK2-related coronavirus as the causative agent of a major swine acute diarrhea syndrome (SADS) outbreak in Southern China.

Your current proposal complements and substantially expands this approach in promising novel and more powerful tools to understand how host immune dynamics and heterogeneity in immune response affect the timing, location, and severity of disease outbreaks in wildlife, and risk of spillover from wildlife to human populations.

I very much looking forward to participating in this initiative and should EcoHealth Alliance be successful in its application for renewal, I agree to participate in activities associated with this project, including contributing expertise, helping identify, locate and interpret relevant data, and participating in partner meetings.

This letter conveys my strong interest and commitment to making this initiative a success. I am excited to be part of the initiative to develop tools to better interrogate seroprevalence data and better predict zoonotic disease emergence. I'm confident that the approach will improve our understanding of the dynamics of infectious diseases and have wide application in public health, and I look forward to working with EcoHealth Alliance on this project

Yours sincerely,

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RESOURCE SHARING PLAN

Data Sharing Plan: Data will be available to the public and researchers without cost or registration, and be released under a CC0 license, as soon as related publications are in press. Data will be deposited for in long-term public scientific repositories – all sequence data will be made publicly available via GenBank, species location data via the Knowledge Network for Biodiversity, and other data will be deposited in appropriate topic-specific or general repositories. Computer code for modeling and statistical analysis will be made available on a code-hosting site (GitHub), and archived in the Zenodo repository under an open-source, unrestrictive MIT license. Limited human survey and clinical data will be released following anonymization and aggregation per IRB requirements. Publications will be released as open-access via deposition to PubMed commons.

Viral isolates will remain at the Wuhan Institute of Virology initially. Isolates, reagents and any other products, should they be developed, will be made available to other NIH-funded researchers via applicable Wuhan Institute of Virology and EcoHealth Alliance Material Transfer Agreements and/or licensing agreements.

Sharing Model Organisms: We do not anticipate the development of any model organisms from this study. Should any be developed, they will be made available to other NIH-funded researchers via applicable Wuhan Institute of Virology and EcoHealth Alliance Material Transfer Agreements and/or licensing agreements.

Genomic Data Sharing: We anticipate obtaining genetic sequence data for 100s of novel coronavirus genotypes, including RNA-dependent RNA polymerase (RdRp) and Spike genes for all strains/genotypes. In addition, we will generate full viral genomes for a subset of the bat SARSr-CoVs that we identify. All sequence data will be deposited in the NIH genetic sequence database, GenBank. We will ensure that all meta-data associated with these sequences, including collection locality lat/long, species-level host identification, date of collection, and sequencing protocols will also submitted. The genotype data will be made publicly available no later than the date of initial publication or six months after the receipt of final sequencing data, whichever comes first. We anticipate sequence generation will occur over the 5 year proposed project period.

Genome Wide Association Studies (GWAS): Not applicable.

Rigor and Authentication of Key Biological and/or Chemical Resources

Our project aims to understand the risk of bat SARS related coronavirus (SARSr-CoV) disease emergence in people, and will use some non-standard biological and chemical resources that require validation and authentication. We will construct chimeric SARSr-CoVs using a WIV1 backbone and the S genes of selected SARSr-CoV strains, and assess capacity to infect hACE2, bACE2 and cACE2 Vero cells, HeLa cells, primary human airway epithelial cells, and potentially CaCo cells for HKU3r-CoVs (which have not yet been cultured in human cell lines and may use intestinal epithelium in nature). We will then conduct experimental infections in hACE2 transgenic mice to assess pathogenicity and clinical signs. Each of these methods have been previously validated and published by our collaborative research team, as highlighted in the research proposal. Each laboratory-based research partner in our project (including University of North Carolina (UNC), Wuhan Institute of Virology (WIV), Institute of Pathogen Biology (IPB) at Chinese Academy of Medical Sciences, and Duke National University of Singapore (DukeNUS)) each have specialized strategies to oversee the authentication of key biological resources, reagents and chemical resources. EcoHealth Alliance will actively engage with each partner to ensure that the highest quality science, public accountability, and social responsibility in the conduct of science are maintained throughout. The overall goal is to ensure that the underlying scientific foundation of the project from conception to completion is scientifically sound. To ensure scientific rigor (e.g., determining group sizes, analyzing anticipated results, reducing bias, ensuring independent and blinded measurements, improving precision and reducing variability including or excluding research subjects, and managing missing data), EcoHealth Alliance will review scientific approaches and outcomes throughout the duration of the award. We will ensure that experimental designs will include considerations of sex as a “Relevant Biological Variables” in all studies involving human subjects or vertebrate animals. Unless otherwise specified and justified, all experiments will include male and females. UNC and WIV will implemented an “audit trail” that tracks animals used in experimental investigations (Aim 3) from parents, through birth, shipment, experimentation, results, QC and analyses, providing outside researchers the ability to track experiments from conception through publication.

Cells.
Early passage primary human airway epithelial cells are a key reagent for the proposed studies. Human lung cells are derived from donors of both sexes and from all ages and ethnic groups. Care is taken during cell isolation to only handle one human organ at a time. Similarly, primary cell populations are handled carefully, only one donor cell type from a single donor at a time to avoid any mixing. The cells are observed to exhibit well-described prototypical characteristics of human primary lung cells in cell type specific medias in culture. For quality control, the cells are cultured in antibiotic free media to test for bacterial and fungal microbial contamination and are subjected to mycoplasma testing. Once the epithelial cells are grown as polarized and differentiated monolayers, a representative sample is subject to quality control histological analysis of cell morphology and Short Terminal Repeat (STR) marker profiling by the UNC Lineberger Cancer Center’s Tissue Culture Facility (TCF). Routine evaluations for mycoplasma contamination are routinely performed in the laboratory.

- Certain experiments also employ immortal cell lines. Cell lines are obtained from the ATCC, or from the TCF. The TCF maintains cell lines, utilizing STR marker profiling and records of authentication are available. New cell lines not available directly from the TCF can be authenticated through the STR marker service provided by the TCF. Cell lines are routinely evaluated for mycoplasma contamination.
- When receiving cell lines, lab members initially maintain isolation and keep them isolated from other authenticated cell lines until mycoplasma testing and STR marker profiling is performed. All cell lines must be authenticated before commencing experimental work with them.
- Records are maintained for each of the cell lines regarding 1) the origin of the cell lines; 2) when they were resuscitated; 3) number of passages; 4) all test results; 5) any unique distinguishing growth behavior; and 6) any known genetic features.
- Cells that have been passaged for 6 months after receipt or from resuscitation will be re-authenticated, or a new vial of the working stock will be thawed.
- Lab members routinely examine cultured cell morphology by phase microscopy and monitor the growth characteristics in culture. New vials of the working stock are thawed if deviation from the baseline is

observed.

- Mycoplasma contamination is re-checked whenever cells are extensively passaged to create new stocks.

Animals (Mice)

- **Rodent genotyping for mouse strain genetic validation**. Inbred mouse strains are an invaluable tool for biomedical research, and hACE2 transgenic mice represent a key aspect of Aim 3 to assess pathogenicity and clinical signs for SARSr-CoVs. To ensure that the genetic background of all mice used within this program is known and when applicable they are part of a known inbred strains, we will genotype each mouse strain used within this program on the appropriate MUGA platform (Morgan, AP et.al., G3 2016, Dec 18). The most recent iteration of this state of the art genotyping array contains over 140,000 markers and can be used to precisely determine the genetic background at the substrain level and the precise location (at <1 megabase resolution) of genomic regions derived from different mouse inbred strains. In this way, the identity and genomic integrity of all mice used within these studies will be ensured. As new diagnostic assays become available, we will assess their utility and cost effectiveness the different MUGA arrays and implement them as appropriate.
- Furthermore, for each mutant mouse strain used within the project, positive diagnoses of the mutation will be assessed for each cohort of experimental animals with a diagnostic validated PCR assay or Sanger sequencing diagnostic to ensure proper results.

Recombinant and Wildtype Viruses and Mutant Derivatives.

- Recombinant and wildtype viruses contain unique marker mutations that allow for distinguishing strains and mutation profiles, using a combination of full genome sequencing, reverse transcription-polymerase chain reaction (RT-PCR) or RT-PCR restriction fragment length polymorphism analyses (RT-PCR RFLP). Our group has developed defined primer pairs to distinguish between SARS-CoV and SARS-related bat coronaviruses as well as MERS-CoV and MERS-related bat coronaviruses. All viruses will be validated and certified pure of contaminating viruses prior to use or shipment to other laboratories.